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<input type="checkbox"/>	L1	kusters.in. or cattoli.in.	1250
<input type="checkbox"/>	L2	L1 and felis	4
<input type="checkbox"/>	L3	felis.ti,ab,clm. not l2	239
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	<i>DB=EPAB; PLUR=YES; OP=OR</i>		
<input type="checkbox"/>	L5	WO-9911284-A1.did.	1
<input type="checkbox"/>	L6	WO-9911284-A1.did.	1

END OF SEARCH HISTORY

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☐ 4. EP 1176192A. Novel *Helicobacter felis* urease X and Y subunit polypeptides, useful in the diagnosis of *Helicobacter felis* infections and in the preparation of vaccines. CATTOLI, G, et al. A61K038/00 A61K039/00 A61K039/106 A61K039/118 A61K039/12 A61K039/175 A61K039/23 A61K039/235 A61K039/38 A61K039/39 A61K039/395 A61K048/00 A61P001/04 A61P031/04 C07K014/195 C07K014/205 C07K016/12 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N009/80 C12N015/09 C12N015/52 C12N015/55 C12Q001/68 G01N033/15 G01N033/50 G01N033/53 G01N033/566 G01N033/569 G01N033/68 C12N009/80 C12Q001/68 C12R001:01 C12R001:01.

DOCUMENT-IDENTIFIER: US 6290962 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Urease-based vaccine and treatment for helicobacter infection

Abstract Text (1):

Method of eliciting in a mammalian host a protective immune response to Helicobacter infection and treatment of Helicobacter infection by administering to the host an immunogenically effective amount of a Helicobacter urease or urease subunits as antigen. Vaccine compositions are also provided.

CLAIMS:

1. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease.
3. The method of claim 1, wherein said Helicobacter urease is Helicobacter pylori urease.
21. A method of treating Helicobacter infection in a mammal, said method comprising administering to a mucosal surface of said mammal a therapeutically effective amount of a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease.
23. The method of claim 21, wherein said Helicobacter urease is Helicobacter pylori urease.
41. A vaccine composition consisting essentially of a polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.
42. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter pylori urease.
43. The vaccine composition of claim 41, wherein said Helicobacter urease is Helicobacter felis urease.
54. A vaccine composition comprising a purified polypeptide comprising an A subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.
56. A vaccine composition consisting essentially of a polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a mucosal adjuvant.
57. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter pylori urease.
58. The vaccine composition of claim 56, wherein said Helicobacter urease is Helicobacter felis urease.
69. A vaccine composition comprising a purified polypeptide comprising a B subunit of a naturally occurring Helicobacter urease, and a polypeptide of the labile toxin of Escherichia coli.



US006290962B1

(12) **United States Patent**  
**Michetti et al.**

(10) **Patent No.:** **US 6,290,962 B1**  
**(45) Date of Patent:** **\*Sep. 18, 2001**

(54) **UREASE-BASED VACCINE AND  
TREATMENT FOR HELICOBACTER  
INFECTION**

WO 93/16723 2/1993 (WO).  
WO 94/09823 11/1994 (WO).  
WO 95/03824 2/1995 (WO).

(75) **Inventors:** **Pierre Michetti; Irène  
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Jean-Pierre Kraehenbuhl, Riva;  
Emilia Saraga, Lausanne, all of (CH)**

(73) **Assignee:** **OraVax, Inc., Cambridge, MA (US)**

(\*) **Notice:** Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-  
claimer.

(21) **Appl. No.:** **08/200,346**

(22) **Filed:** **Feb. 23, 1994**

#### **Related U.S. Application Data**

(63) Continuation-in-part of application No. 08/085,938, filed on  
Jul. 6, 1993, now Pat. No. 5,972,336, which is a contin-  
uation-in-part of application No. 07/970,996, filed on Nov. 3,  
1992, now abandoned.

(51) **Int. Cl.<sup>7</sup>** ..... **A61K 39/00; A61K 38/46;  
A61K 31/70; A61K 39/385**

(52) **U.S. Cl.** ..... **424/185.1; 424/234.1;  
424/184.1; 424/192.1; 424/193.1; 424/197.1;  
424/261.1; 424/280.1; 424/278.1; 424/94.6;  
424/282.1; 424/203.1; 514/41; 514/234.5**

(58) **Field of Search** ..... **424/234.1, 192.1,  
424/193.1, 197.11, 261.1, 278.1, 280.1,  
185.1, 184.1, 94.6, 282.1, 203.1; 514/41,  
234.5**

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*Primary Examiner*—Nita Mannfield

(74) *Attorney, Agent, or Firm*—Clark & Elbing LLP

(57) **ABSTRACT**

Method of eliciting in a mammalian host a protective  
immune response to *Helicobacter* infection and treatment of  
*Helicobacter* infection by administering to the host an  
immunogenically effective amount of a *Helicobacter* urease  
or urease subunits as antigen. Vaccine compositions are also  
provided.

**72 Claims, 6 Drawing Sheets**

DOCUMENT-IDENTIFIER: US 6258359 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NO: 22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NO: 20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NO: 20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NO: 22-26);

CLAIMS:

4. A composition comprising purified monoclonal antibodies directed against the following peptides:

a) at least one urease polypeptide of *Helicobacter felis* or *Helicobacter pylori* selected from the group consisting of UreA, UreB, UreE, UreF, UreG, UreH, UreI, and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment; and

b) at least one polypeptide of *Helicobacter felis* or *Helicobacter pylori* selected from the group consisting of HspA (SEQ ID NO: 29), HspB (SEQ ID NO: 30), and fragments thereof, wherein said fragment is also recognized by an antibody directed against the full length polypeptide corresponding to that fragment.

5. The composition of claim 4, wherein said urease polypeptide is UreA of *Helicobacter pylori*.

6. The composition of claim 4, wherein said urease polypeptide is UreB of *Helicobacter pylori*.

7. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of *Helicobacter pylori*.

8. The composition of claim 4, wherein said urease polypeptide is UreA of *Helicobacter felis*.

9. The composition of claim 4, wherein said urease polypeptide is UreB of *Helicobacter felis*.

10. The composition of claim 4, wherein said urease polypeptides are UreA and UreB of *Helicobacter felis*.



US006258359B1

(12) **United States Patent**  
**Labigne et al.**

(10) **Patent No.:** **US 6,258,359 B1**  
**(45) Date of Patent:** **Jul. 10, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST  
 HELICOBACTER INFECTION,  
 POLYPEPTIDES FOR USE IN THE  
 COMPOSITIONS, AND NUCLEIC ACID  
 SEQUENCES ENCODING SAID  
 POLYPEPTIDES**

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 Sebastian Suerbaum, Veitshöchheim  
 (DE); Richard L. Ferrero, Paris;  
 Jean-Michel Thiberge, Plaisir, both of  
 (FR)

(73) **Assignee:** Institut Pasteur, Paris (FR)

(\*) **Notice:** Subject to any disclaimer, the term of this  
 patent is extended or adjusted under 35  
 U.S.C. 154(b) by 0 days.

(21) **Appl. No.:** 08/466,248

(22) **Filed:** Jun. 6, 1995

#### Related U.S. Application Data

(60) Division of application No. 08/447,177, filed on May 19,  
 1995, now abandoned, which is a continuation-in-part of  
 application No. 08/432,697, filed on May 2, 1995, which is  
 a continuation-in-part of application No. PCT/EP94/01625,  
 filed on May 19, 1994.

#### (30) Foreign Application Priority Data

May 19, 1993 (EP) ..... 93 401 309  
 Nov. 19, 1993 (WO) ..... PCT/EP93/03259

(51) **Int. Cl.<sup>7</sup>** ..... A61K 39/395; A61K 39/40;  
 C07K 1/00; C07K 16/00

(52) **U.S. Cl.** ..... 424/141.1; 424/150.1;  
 424/163.1; 424/164.1; 530/350; 530/388.1;  
 530/388.2; 530/388.4

(58) **Field of Search** ..... 424/234.1, 141.1,  
 424/150.1, 163.1, 164.1; 514/2; 530/350,  
 388.1, 388.2, 388.4

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*Primary Examiner*—Albert Navarro

(74) *Attorney, Agent, or Firm*—Finnegan, Henderson,  
 Farabow, Garrett and Dunner

#### (57) ABSTRACT

There is provided an immunogenic composition capable of  
 inducing protective antibodies against Helicobacter infec-  
 tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide  
 from *Helicobacter pylori* (SEQ ID NO: 22,26), or a  
 fragment thereof, said fragment being recognized by  
 antibodies reacting with *Helicobacter felis urease*  
 (SEQ ID NO: 20-21), and/or at least one sub-unit of a  
 urease structural polypeptide from *Helicobacter felis*  
 (SEQ ID NO: 20-21), or a fragment thereof, said  
 fragment being recognized by antibodies reacting with  
*Helicobacter pylori urease* (SEQ ID NO: 22-26);
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from  
 Helicobacter, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-  
 genic compositions is also provided.

20 Claims, 36 Drawing Sheets

**\*\* See image for Certificate of Correction \*\***

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis urease*, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori urease*;

## CLAIMS:

10. Proteinaceous material comprising a fusion protein, wherein the fusion protein comprises at least one *Helicobacter* HspA or a fragment thereof as defined in any one of claims 6-9 and at least one polypeptide selected from the group consisting of

a *Helicobacter pylori urease* structural polypeptide or fragment thereof, wherein said fragment is recognized by antibodies to *H. felis urease*, and

a *Helicobacter felis urease* structural polypeptide or immunogenic fragment thereof.

11. An immunogenic composition, which induces antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, and a heat shock protein (Hsp) from *Helicobacter* or a fragment thereof, wherein the Hsp protein is HspA or HspB and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), and wherein said fragment has at least 6 amino acids and is immunogenic.

16. An immunogenic composition, capable of inducing antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, further comprising at least one heat shock protein (Hsp) from *Helicobacter*, wherein the Hsp protein is HspA, HspB, or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), or a fragment thereof, wherein said fragment has at least 6 amino acids and is capable of generating antibodies.



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(12) **United States Patent**  
Labigne et al.

(10) Patent No.: **US 6,248,330 B1**  
(45) Date of Patent: **\*Jun. 19, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST  
HELICOBACTER INFECTION,  
POLYPEPTIDES FOR USE IN THE  
COMPOSITIONS, AND NUCLEIC ACID  
SEQUENCES ENCODING SAID  
POLYPEPTIDES**

(75) Inventors: **Agnes Labigne**, Bures sur Yvette (FR);  
**Sebastien Suerbaum**, Bochum (DE);  
**Richard L. Ferrero**, Paris;  
**Jean-Michel Thiberge**, Plaisir, both of  
(FR)

(73) Assignee: **Institut Pasteur**, Paris (FR)

(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-  
claimer.

(21) Appl. No.: **08/432,697**

(22) Filed: **May 2, 1995**

#### Related U.S. Application Data

(63) Continuation-in-part of application No. PCT/EP94/01625,  
filed on May 19, 1994.

#### (30) Foreign Application Priority Data

May 19, 1993 (EP) ..... 93401309  
May 19, 1994 (WO) ..... PCT/EP94/03259

(51) Int. Cl.<sup>7</sup> ..... **A61K 39/00**

(52) U.S. Cl. .... **424/192.1; 424/234.1;**  
424/184.1; 435/6; 435/69.1

(58) Field of Search ..... 424/234.1, 184.1,  
424/203.1, 192.1; 435/6, 7.21

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Assistant Examiner—Ginny Allen Portner

(74) Attorney, Agent, or Firm—Finnegan, Henderson,  
Farabow, Garrett & Dunner

#### (57)

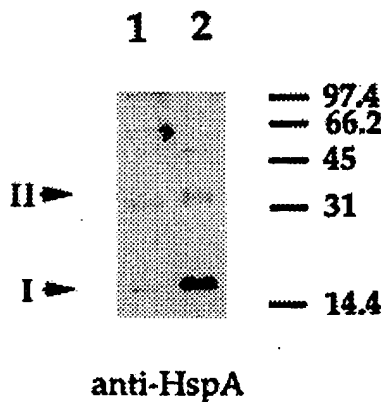
#### ABSTRACT

There is provided an immunogenic composition capable of  
inducing protective antibodies against *Helicobacter* infec-  
tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide  
from *Helicobacter pylori*, or a fragment thereof, said  
fragment being recognized by antibodies reacting with  
*Helicobacter felis* urease, and/or at least one sub-unit of  
a urease structural polypeptide from *Helicobacter felis*,  
or a fragment thereof, said fragment being recognized  
by antibodies reacting with *Helicobacter pylori* urease;
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from  
*Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-  
genic compositions is also provided.

**16 Claims, 36 Drawing Sheets**



DOCUMENT-IDENTIFIER: US 6248330 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Immunogenic compositions against helicobacter infection, polypeptides for use in the compositions, and nucleic acid sequences encoding said polypeptides

Abstract Text (2):

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis urease*, and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis*, or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori urease*;

CLAIMS:

10. Proteinaceous material comprising a fusion protein, wherein the fusion protein comprises at least one *Helicobacter* HspA or a fragment thereof as defined in any one of claims 6-9 and at least one polypeptide selected from the group consisting of

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11. An immunogenic composition, which induces antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, and a heat shock protein (Hsp) from *Helicobacter* or a fragment thereof, wherein the Hsp protein is HspA or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), and wherein said fragment has at least 6 amino acids and is immunogenic.

16. An immunogenic composition, capable of inducing antibodies against *Helicobacter* infection, comprising at least one sub-unit of a purified, synthetic, or recombinant *Helicobacter felis urease* structural polypeptide selected from the group of polypeptides consisting of SEQ ID NO: 20 and SEQ ID NO: 21, further comprising at least one heat shock protein (Hsp) from *Helicobacter*, wherein the Hsp protein is HspA, HspB, or HspA and HspB encoded by the HspA/HspB genes of plasmid pILL689 (CNCM I-1356), or a fragment thereof, wherein said fragment has at least 6 amino acids and is capable of generating antibodies.



US006248330B1

(12) **United States Patent**  
Labigne et al.

(10) Patent No.: **US 6,248,330 B1**  
(45) Date of Patent: **\*Jun. 19, 2001**

(54) **IMMUNOGENIC COMPOSITIONS AGAINST  
HELICOBACTER INFECTION,  
POLYPEPTIDES FOR USE IN THE  
COMPOSITIONS, AND NUCLEIC ACID  
SEQUENCES ENCODING SAID  
POLYPEPTIDES**

(75) Inventors: **Agnes Labigne, Bures sur Yvette (FR);  
Sebastien Suerbaum, Bochum (DE);  
Richard L. Ferrero, Paris;  
Jean-Michel Thlberge, Plaisir, both of  
(FR)**

(73) Assignee: **Institut Pasteur, Paris (FR)**

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U.S.C. 154(b) by 0 days.

This patent is subject to a terminal dis-  
claimer.

(21) Appl. No.: **08/432,697**

(22) Filed: **May 2, 1995**

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424/184.1; 435/6; 435/69.1**

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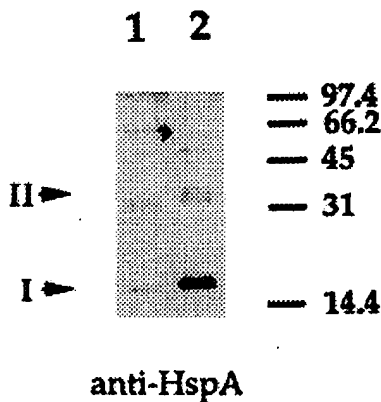
#### (57) ABSTRACT

There is provided an immunogenic composition capable of  
inducing protective antibodies against *Helicobacter* infec-  
tion characterized in that it comprises:

- i) at least one sub-unit of a urease structural polypeptide  
from *Helicobacter pylori*, or a fragment thereof, said  
fragment being recognized by antibodies reacting with  
*Helicobacter felis* urease, and/or at least one sub-unit of  
a urease structural polypeptide from *Helicobacter felis*,  
or a fragment thereof, said fragment being recognized  
by antibodies reacting with *Helicobacter pylori* urease;
- ii) and/or, a heat shock protein (Hsp), or chaperonin, from  
*Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immuno-  
genic compositions is also provided.

16 Claims, 36 Drawing Sheets



DOCUMENT-IDENTIFIER: US 6005090 A

TITLE: Treatment and prevention of helicobacter infection

Abstract Text (1):

An antigenic preparation for use in the treatment or prevention of Helicobacter infection in a mammalian host, comprises the catalase enzyme of Helicobacter bacteria, particularly the catalase enzyme of H. pylori or H. felis, or an immunogenic fragment thereof.

CLAIMS:

8. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

9. A vaccine composition for use in the treatment or prevention of Helicobacter infection in a mammalian host, consisting essentially of an immunologically effective amount of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

10. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

11. A method for the treatment or prevention of Helicobacter infection in a mammalian host, which comprises administration to said host of an immunologically effective amount of an antigenic preparation consisting essentially of Helicobacter pylori catalase, together with Helicobacter urease and Helicobacter lipopolysaccharide, and further together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

12. A method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase and an additional Helicobacter antigen, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent, and wherein said additional antigen is Helicobacter urease or Helicobacter lipopolysaccharide.

13. Method of producing a vaccine, comprising the step of bringing an antigenic preparation into a form suitable for administration to a mammal, wherein said preparation consists essentially of Helicobacter pylori catalase, and Helicobacter urease and Helicobacter lipopolysaccharide, together with a mucosal adjuvant and a pharmaceutically acceptable carrier or diluent.

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/GB98/02631 <b>(22) International Filing Date:</b> 2 September 1998 (02.09.98) <b>(30) Priority Data:</b> 9718616.7 2 September 1997 (02.09.97) GB <b>(71) Applicant (for all designated States except US):</b> QUEEN MARY & WESTFIELD COLLEGE [GB/GB]; Mile End Road, London E1 4NS (GB). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> TABAQCHALI, Soad [GB/GB]; 9 Kent Terrace, London NW1 4RP (GB). WILKS, Mark [GB/GB]; 27 Grayling Road, London N16 0BL (GB). <b>(74) Agent:</b> NACHSHEN, Neil, Jacob; D. Young & Co., 21 New Fetter Lane, London EC4A 1DA (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> ORAL VACCINE  <b>(57) Abstract</b>  The present invention relates to a vaccine comprising a <i>Lactobacillus</i> species that contains a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease humoral and/or cellular immune response upon administration to a mammalian species.		

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ORAL VACCINE

The present invention relates to modified Lactobacilli and their use in the  
5 treatment of gastric disorders.

Gastric disorders such as gastritis, peptic ulcer disease etc. have recently been  
discovered to be due to the presence of Helicobacter strains in the stomach,  
particularly Helicobacter pylori. Consequently, much effort has been invested in  
10 the research for agents that will minimise the effect of Helicobacter strains and  
specifically H. pylori.

The results of this research include many chemical and biological agents that are  
designed to inhibit the bacteria, its activity and colonisation of the stomach.  
15 Although H pylori has been shown to be susceptible to many agents in in vitro  
tests, in vivo eradication has not often been achieved (Czinn SJ et al., Infect.  
Immunol. (1991) 59 2359-2363).

Urease is the most prominent protein component of Helicobacter pylori and it has  
20 been proposed that urease is produced in order to hydrolyse urea thus increasing  
the pH of the environment to one favourable to colonisation by Helicobacter  
(Moblely HLT et al., Microbiol Rev (1995) 59 451-480).

Thus, attempts have been made to eliminate or control H pylori by the  
25 administration of urease through various means. Pallen MJ & Clayton CL (The  
Lancet (1990) 336 186-7) suggested oral immunisation using plant urease, an idea  
carried out by Chen M et al. (FEMS Microbiol Lett. (1994) 116 245-250) using  
jack bean urease and a cholera toxin adjuvant. This and many other related  
strategies are described in Mobley HLT et al. supra.

30

International Patent Applications Publication Nos. WO95/22987 and  
WO96/33732 both describe urease based vaccines that utilise recombinant urease.

In both cases the cloning of the urease gene or fragments thereof, has been utilised as a method of producing sufficient urease to be administered together with a pharmaceutical carrier. Neither application discloses the oral administration of the expression vector itself for expression to occur in vivo.

5

The hope offered in utilising urease as a vaccine has not yet been fully realised due to difficulties in designing an efficient system for delivering sufficient urease to the desired site to stimulate a humoral and/or cellular immune response, in particular to initiate the production of secretory IgA, without undesirable or  
10 unwanted side effects to the recipient, due for example to an adjuvant.

The present invention therefore aims to provide a vaccine that is capable of delivering sufficient urease to the desired site such as to control/eradicate and/or prevent Helicobacter colonisation and thus treat Helicobacter related diseases.

15

Accordingly, the present invention relates to a vaccine comprising a Lactobacillus species that contains a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease humoral and/or cellular immune response upon administration to a mammalian species, preferably initiating the production of  
20 secretory IgA.

Suitable Lactobacillus species include any species or subspecies such as Lactobacillus delbrueckii and subspecies bulgaricus and lactis, Lactobacillus GG, a strain of L casei subspecies rhamnosus (Goldin et al., (1992) Dig Dis and  
25 Sciences 37 121-128), L fermentum, L. planatarum, L paraplantarum, L pentosus, L coryniformis, L casei, L paracasei, L brevis, L leichmannii and strains of Lactobacillus isolated from intestinal flora such as Lactobacillus rhamnosus 901. Lactobacillus rhamnosus 901 is of particular benefit as it possesses resistance to both acid and bile juices. In accordance with the present invention, the  
30 Lactobacillus spp is been modified such that the organism contains a nucleotide sequence either on a plasmid or in a chromosome capable of expressing a urease peptide as defined above.

Preferably, the Lactobacillus strain used is L. planatarum. More preferably, the strain of L. planatarum is selected from L. planatarum NCIMB 8826, NC4Ts1, NC7Ts5 or ATCC 8014, most preferably the NCIMB strain 8826 of L.  
5 planatarum is used.

Urease is a protein that is encoded by an array of genes that are comprised of structural, accessory and regulatory subunits. These are all required in order to produce a biologically active enzyme, for example certain subunits are involved  
10 with the incorporation of nickel into the protein structure that is essential for enzymatic activity. As discussed below, the retention of enzymatic activity is not essential for the purposes of the present invention.

The nucleotide sequences of use in the present invention must encode at least a  
15 fragment of urease capable of initiating an anti-urease humoral and/or cellular immune response upon administration to a mammalian species. Such an immune response is defined herein as the ability to initiate the production of anti-urease antibodies, in particular systemic and local IgA, detectable by methods known in the art, preferably by ELISA.

20 Suitable nucleotide sequences that are able to express a urease peptide capable of initiating a anti-urease humoral and/or cellular immune response include those that encode the whole urease protein, fragments thereof, homologs or analogs thereof. The full nucleotide sequences of H. pylori urease, including the genes  
25 encoding the structural subunits A and B (ureA and ureB), were reported in Clayton CL et al. Nucleic Acid Research (1990) 18 362 and these sequences may be used together or individually. It should be noted that the Helicobacter spp ureA peptide represents a fusion of ureA and ureB peptides in other microorganisms whereas the Helicobacter spp UreB peptide and H pylori UreH  
30 peptide are homologous to UreC and UreD peptides in other bacteria. Mobley HLT et al., Microbiol Revs, (1995), vol 59, pages 451-480, provides a detailed description of known urease sequences and any of these sequences in whole or

part that is capable of initiating an anti-urease immune response as defined above, may be used in the present invention. A non-exhaustive list of nucleotide sequences encoding urease is given in Table 1. As used hereinafter, all reference to urease subunits relate to the nomenclature used for H. pylori in Clayton CL et al. (supra).

Table 1

Species	Genes Sequenced	GenBank Accession No.	References
Complete gene cluster sequenced			
<i>Bacillus</i> sp. strain TB-90 H.	ureABCEFGDHI	D14439	Maeda M, <i>et al</i> 1994, J. Bacteriol, 176:432-442
<i>H. pylori</i> <sup>+</sup>	ure, ABIEFGH	M84338, X57132, X17079, M60398	Clayton C, <i>et al</i> 1990, Nucleic Acids Res. 18:362, Cussac V, <i>et al</i> 1992, J. Bacteriol, 174: 2466-2473., Labigne A, <i>et al</i> 1991, J. Bacteriol, 173:1920-1931.
<i>K. aerogenes</i>	ureDABCEFG	M55391, M36068	Lee M.H, <i>et al</i> 1992, J. Bacteriol, 174:4324-4330.
<i>P. mirabilis</i>	ureRDABCEFG	M31834, Z18752, Z21940	Mulrooney S.B. <i>et al</i> 1990, J. Bacteriol, 172:5837-5843.
<i>Y. enterocolitica</i>	ureABCEFGD	Z18865, L24101	Jones B.D, <i>et al</i> 1989, J. Bacteriol, 171:6414-6422.
Complete Sequence for Selected Genes			Nicholson E.B, <i>et al</i> 1993, J. Bacteriol, 175:465-473.
<i>B. pasteurii</i>	ureABC	X78411	Sriwanthana B, <i>et al</i> 1993, G. Gene, 129:103-106.
<i>H. felis</i>	ureAB	X69080	de Koning-Ward T.F <i>et al</i> 1994, Gene 145:25-32
<i>H. heilmannii</i>	ureAB	L25079	Skumilk, M <i>et al</i> 1993, Immun. 61:2498-2504.
<i>K. pneumoniae</i>	ureDA	L07039	
<i>L. fermentum</i>	ureABC	D10605	Morsdorf G, <i>et al</i> 1994, <i>Bacillus pasteurii</i> GenBank accession No. x78411
<i>P. vulgaris</i>	ureABC	X51816	Ferrero, R.L., <i>et al</i> 1993, Mo. Microbiol, 9:323-333.
<i>R. meliloti</i>	ureDABC	S69145	Solnick J.V <i>et al</i> 1994, Infect. Immun, 62:1631-1638
<i>S. xyloosus</i>	ureABC	X74600	Collins C.M. <i>et al</i> 1993, Mol.Microbiol. 8:187-198
<i>U. urealyticum</i>	ureABC	X51315	Suzuki K. <i>et al</i> 1992, <i>Lactobacillus fermentum</i> JCM5869, GenBank accession No. D10605
			Morsdorf G <i>et al</i> 1990, FEMS Microbiol, Lett: 66:67-74
			Miksch G. 1994, FEMS Microbiol, Lett: 124:185-190
			Miksch G, <i>et al</i> 1994, Mol. Gen. Genet. 242:539-550
			Jose, J, <i>et al</i> 1994, Arch. Microbiol, 161:384-392
			Blanchard, A. 1990
			Mol. Microbiol 4:669-678

The nucleotide sequences of use in the present invention, may be used alone or as part of a larger sequence encoding a fusion protein comprising a urease moiety or peptides homologous with urease. For example, urease B subunit may be expressed as a chimeric protein together with the cholera toxin B subunit by the insertion into a Lactobacillus spp of the urease B subunit nucleotide sequence linked to the cholera toxin B subunit nucleotide sequence.

As discussed above, nucleotide sequences that are homologous to a known urease encoding sequence may be used in the present invention. Preferably, such a sequence bears at least 70% homology to the H. pylori nucleotide sequence, more preferably at least 80% and most preferably at least 90 or 95% homology. For example, urease such as that encoding for jack bean urease bears about 70% homology with H pylori urease.

It is preferable to use a nucleotide sequence derived from H. pylori, but alternative sources of suitable nucleotide sequences include other Helicobacter species such as H felis, H heilmannii or Morganella morganii. Preferably, the nucleotide sequence encodes for at least the urease A or B subunits or A and B subunits. More preferably the nucleotide sequence encodes for at least the urease B subunit.

The urease peptide may possess urease activity or alternatively be devoid of such activity.

The nucleotide sequence may be inserted into the Lactobacillus genome or Lactobacillus plasmid as a single entity or in clusters linked together such as to produce a multimeric protein comprising for example, from two to eight urease A subunits and from two to eight urease B subunits. Within this embodiment, when more than one subunit is inserted into Lactobacillus, they may be inserted in equal or unequal numbers.

In preparation of a vaccine of the present invention, the appropriate nucleotide sequence capable of expressing a urease peptide may be amplified and isolated from a suitable source using PCR, fused with a Lactobacillus or Lactococcus promoter, ligated into a vector, plasmid or transposon such as Tn916 and then  
5 introduced into the Lactobacillus strain by for example, electroporation. An example of a suitable method for inserting a foreign gene into a Lactobacillus genome is given in European Patent Application 603416A, wherein a DNA cassette is formed comprising Lactobacillus DNA fragments from upstream and down stream of the proposed chromosomal integration site that are ligated to  
10 either end of the nucleotide sequence to be inserted, and the cassette inserted into pAM $\beta$ 1 plasmid which is used as an integration plasmid. This plasmid is then introduced into a Lactobacillus spp such as L delbueckii by conjugal transfer to obtain transconjugants by integration into chromosomal DNA, that exhibit erythromycin resistance (originating from the plasmid). The subculturing is then  
15 repeated to generate subclones that become sensitive to erythromycin as a result of losing the relevant sequence from pAM $\beta$ 1 and from these subclones selecting those that contain the inserted nucleotide sequence.

Further methods of inserting nucleotide sequences into L plantarum include those  
20 disclosed in Hols P et al., (Microbiol (1997) 143, 2733-2741) that describes the insertion of sequences encoding the N-terminal portion of the Streptococcus pyogenes M6 portein fused to an epitope of the HIV gp41 protein into L plantarum NCIMB 8826, and Cosby WM et al., (Plasmid (1989) 22, 236-243) that describes the electroporation of the pTV1Ts temperature sensitive plasmid  
25 carrying the macrolide-lincosamide-streptogramin B resistance transposon Tn917 into L plantarum.

The transformed Lactobacillus may be used directly in the vaccine composition. Thus, an advantage of the present invention is that the vaccine is easily prepared  
30 not requiring the lengthy purification involved when handling recombinant proteins. The vaccine may be given alone or preferably included in foodstuffs that already contain a Lactobacillus spp such as yoghurt, fermented milk drink or

cheese etc. as the sole source of Lactobacillus or in addition to the existing strains.

5 The vaccines of the present invention may be used in the treatment of gastrointestinal (duodenal) disorders including gastritis, peptic ulcer disease including both gastric and duodenal ulcers, gastric cancer, chronic dyspepsia with severe erosive gastroduodenitis, refractory ulcer dyspepsia, intestinal metaplasia, low grade MALT lymphoma, Helicobacter infection, Helicobacter pylori infection and Helicobacter felis infection.

10

The vaccines may further contain pharmaceutically acceptable excipients such as adjuvants, solvents, preservatives, stabilisers and the like. Furthermore, the vaccine may additionally contain other pharmacologically active ingredients such as antibiotics, antisecretory agents and bismuth salts.

15

A further aspect of the present invention relates to transformed Lactobacillus spp. containing a nucleotide sequence that encodes a urease peptide as defined above capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.

20

In use, the vaccine may be administered by methods known in the art. Thus it may be given by intravenous (I.V.), intramuscular (I.M.), subcutaneous (S.C.), intradermal (I.D.) or oral routes and the like. The dose is administered at least once. Subsequent doses may be administered as indicated. It is a particular  
25 advantage of the present invention that the vaccine may be administered orally. This advantage is assisted by the incorporation of Lactobacillus spp being incorporated into foodstuffs as discussed above. Administration of the vaccine may involve a priming dose, optionally by an alternative route to the main

dosage. For example, when the vaccine is to be administered orally, a subcutaneous priming dosage may be administered.

In providing a mammal, preferably a human, with the vaccine of the present  
5 invention, the dosage will vary depending upon such factors as the mammal's age, weight, height, sex, general medical condition, previous medical history, disease progression and the like.

## EXAMPLES

**Example 1; Transformation of *L. plantarum***

*L. plantarum* NCIMB strain 8826 was readily transformed by electroporation using a variety of plasmids including pNZ12, pNZ17, pNZ10 $\alpha$ 1, pTG3237 (all obtained from Netherlands Institute for Dairy Research (NIZO), Bostbus 20, 6710 BA, Ede, Netherlands). The highest transformation rate was achieved with pNZ10 $\alpha$ 5 where transformants typically arose at a frequency of  $>10^3$  per  $\mu$ g of plasmid DNA.

10

Optimum recovery of plasmid DNA was achieved with *Lactobacillus plantarum* strain NCIMB 8826 and the vector pNZ10 $\alpha$ 5. 13 $\mu$ g DNA/100 ml culture is recovered using a modification of the method suggested by Frere (Letters in Applied Microbiology 18 227-9). In this modification, cell suspensions are incubated at 37°C for one hour and then vortexed for 1 min with an equal volume of glass beads (0.1-0.15 mm diameter) to disrupt the cell wall.

15

Once electroporated into strain 8826, pNZ10 $\alpha$ 5 has been found to be stable as shown by repeated subculture for up to 86 generations.

20

**Example 2; Colonisation studies of *Lactobacillus* spp in BALB/c mice. Results and approach using *Lactobacillus plantarum* strain NCIMB 8826 and the vector pNZ17**

9 BALB/c mice are divided into 3 groups and fed with pNZ17-transformed *Lactobacilli*. Transformants of *Lactobacillus* GG, *Lactobacillus* Sp. 901, and *L. plantarum* 8826 containing pNZ17 are prepared as described in Example 1, and then cultured in skimmed milk.

A single colony of each species of *Lactobacilli* is transferred into 100 ml of MRS (de Man, Rogosa & Sharpe) broth containing chloramphenicol 20 $\mu$ g/ml, respectively. The cultures are incubated overnight at 37°C without shaking.

30

Bacterial suspensions are harvested by centrifugation at 5,000g for 15 min at room temperature, the supernatant decanted and the bacterial pellet resuspended in 20 ml of skimmed milk.

5

Viable counts of each strain are prepared by pipetting 100µl of each bacterial suspension in 900µl MRS broth and then making serial 10-fold dilution. 100µl of each dilution is plated onto MRS agar plates and the number of colonies counted after 48 hours of incubation at 37°C.

10

Typical concentrations of Lactobacilli in skimmed milk were:

Lactobacillus GG:  $3 \times 10^8$  cfu/ml -  $2 \times 10^9$  cfu/ml,

Lactobacillus 901:  $2 \times 10^9$  cfu/ml,

Lactobacillus plantarum NCIMB 8826:  $3 \times 10^9$  cfu/ml.

15

Mice are fed 1 ml of lactobacilli-milk twice daily for 18 days and faeces collected three times a week for culture on MRS agar plates with and without chloramphenicol 20 µg/ml.

20 During feeding, Lactobacilli is detected in the faeces of all mice at high concentrations

Lactobacillus GG  $2 \times 10^7$  cfu/g

Lactobacillus Sp. 901  $1.2 \times 10^7$  -  $1 \times 10^8$  cfu/g

25 Lactobacillus Plantarum 8826  $7.4 \times 10^7$  -  $1.2 \times 10^8$  cfu/g

**24 h after cessation of feeding:**

Lactobacillus GG not detected

Lactobacillus Sp. 901 not detected

30 Lactobacillus plantarum 8826  $2-3 \times 10^8$  cfu/g

**5th day after cessation of feeding**

Lactobacillus plantarum 8826 still detectable at a concentration of  $7 \times 10^2$  cfu/g

**5 Culture of tissue from killed BALB/c mice,**

24h after cessation of feeding

Lactobacillus GG

$5 \times 10^2$  cfu/g small intestine

10  $4 \times 10^3$  cfu/g caecum

$3.5 \times 10^3$  cfu/g colon

Lactobacillus 901

$1.35 \times 10^3$  cfu/g caecum

15  $2.6 \times 10^5$  cfu/g colon

Lactobacillus plantarum 8826

$5 \times 10^3$  cfu/g small intestine

$8.6 \times 10^7$  cfu/g caecum

20  $2.5 \times 10^6$  cfu/g colon

**Example 3; Transformant plasmids****Urease:**

25 DNA encoding the structural subunits of urease gene, ureA and ureB (Clayton CL supra) is amplified by use of the polymerase chain reaction using Pfu *Taq* polymerase to ensure error free amplification. The primers used are;

YR1 : 5' AAGGAT TTAAGGAGCGTTGC 3' and,

YR2 : 5' GATTTCGTTATGTCTTCAAGG 3'

Alternatively, plasmid pTCP3 containing a 2.6kb insert encoding both the 66kDa ureB and 31kDa ureA subunits is used (Clayton CL et al and Infect Immun (1989) 57(2), 623-629). If so, pTCP3 is prepared by transformation into *E. coli* JM109 and digested using TaqI to release the urease encoding fragment.

5

#### **Construction of the secretion plasmid in pNZ10 $\alpha$ 5**

pNZ10 $\alpha$ 5 carries the amyS gene fused to part of the *L. lactis* promoter gene usp45 gene (van Asseldonk et al 1993 Molecular and General Genetics 240, 428-434).

10

A secretion plasmid containing translational fusions between usp45 from *Lactococcus lactis* and the ureA+B or ureB gene is constructed. This encodes a highly secreted protein which maximises exposure to the host's mucosal immune system.

15

Fusion expression is driven by the promoter (-35 and -10) sequences of usp45, its ribosome binding site, translational start site and signal peptide (27 aa from ATG to Ala 27 which is the cleavage site).

20 To construct the urease secretion plasmid, pNZ10 $\alpha$ 5 is cut with PstI and HindIII to provide the first 57bp (19 first aa) of the signal sequence. A synthetic linker containing the final 24bp (8 aa) of the signal sequence and a restriction site is inserted into which the ureA and ureB or ureB only gene derived from PCR products or pTCP3 are cloned.

25

Successful insertion and expression of sequences encoding urease subunits is confirmed by Western blotting as described below.

#### **Example 4; Detection of anti-urease antibodies**

30

Anti-urease antibodies is detected in the sera of *H pylori* infected animals by ELISA as described in Tanaka K et al., Gut (1991) 32, 43-45.

- Microtest III plates (Becton-Dickinson) are coated with urease at a concentration of 0.04 mg/ml in carbonate/bicarbonate buffer pH 9.6. The plates are incubated overnight at 4°C and then washed three times with phosphate buffered saline (PBS) pH 7.4 containing 0.05% (v/v) Tween20 (PBS/T20). Bovine serum albumin (1%w/v) (Sigma) in PBS pH 7.4 (PBS/BSA) is added to the wells to reduce non-specific binding. Plates are incubated for two hours at room temperature, washed three times with PBS/T20, and stored at -20°C until needed. 100µl of mouse serum is diluted 1:50 with PBS/BSA and added to the wells.
- 10 After incubation overnight at 4°C, the plates were washed three times with PBS/T20. 100µl of anti-mouse IgG conjugate diluted according to the manufacturer's instruction is added to the wells. The plates are incubated for two hours at room temperature and washed three times with PBS/T20. A total of 100µl of 1 mg/ml alkaline phosphate substrate (Sigma 104) in substrate buffer pH 15 8.9 consisting of 0.2 M NaCO<sub>3</sub>, 0.2M NaHCO<sub>3</sub>, 0.01M MgCl<sub>2</sub> is added and incubated for one hour at room temperature. The reaction is stopped with the addition of 100µl of 1N NaOH and the colour read spectrophotometrically at 405nm using a Dynex MR1 ELISA reader.
- 20 Highly pure urease for coating the wells may be obtained from H. pylori using the methods described in Icatlo FC et al., J Biol Chem (1998) 273(29), 18130-18138.

- Western blotting, is performed as in Tanaka K et al., supra with the modification that whole cell preparation of the Helicobacter pylori NCTC 11637 or 630 is used. The protein concentration is determined by a modified Lowry technique, protein dissolved in 10% sodium dodecyl sulphate and approximately 25µg of total protein is loaded in each lane and electrophoresed in a Hoefer Transblotter in a discontinuous buffer system at a constant current of 15 mA. Mice sera from vaccinated animals is diluted and tested at dilutions of 1/25 and 1/50.
- 25

CLAIMS

1. A vaccine comprising a Lactobacillus species containing a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.  
5
2. A vaccine according to claim 1, wherein the nucleotide sequence encodes the whole urease protein, fragments, homologs or analogs thereof.
- 10 3. A vaccine according to claim 1 or 2, wherein the nucleotide sequence encodes structural subunits of urease.
4. A vaccine according to any preceding claim, wherein the nucleotide sequence is derived from a Helicobacter species.
- 15 5. A vaccine according to claim 4, wherein the Helicobacter specie is Helicobacter pylori.
6. A vaccine according to any preceding claim, wherein the nucleotide sequence encodes for at least the urease A or B subunits or A and B subunits.  
20
7. A vaccine according to claim 6, wherein the nucleotide sequence encodes for at least the urease B subunit.
- 25 8. A vaccine according to any preceding claim, wherein the nucleotide sequence encodes a fusion protein.
9. A vaccine according to claim 8, wherein the fusion protein contains a cholera toxin B subunit.
- 30 10. A vaccine according to any preceding claim, wherein the Lactobacillus spp is selected from Lactobacillus delbrueckii, subspecies bulgaricus and lactis, L

fermentum, L. planatarum, L. paraplantarum, L. pentosus, L. coryniformis, L. casei,  
L. paracasei, L. brevis, L. lechmannii Lactobacillus GG, a strain of L. casi  
subspecies rhamnosus, Lactobacillus rhamnosus 901 and strains of Lactobacillus  
isolated from intestinal flora.

5

11. A vaccine according to claim 10, wherein the Lactobacillus spp is  
Lactobacillus plantarum.

12. A vaccine according to claim 11, wherein the Lactobacillus plantarum is  
10 NCIMB strain 8826.

13. A vaccine according to any preceding claim capable of initiating a cellular  
and humoral anti-urease immune response.

15 14. A vaccine according to any preceding claim capable of initiating  
production of secretory IgA.

15. Use of a vaccine as defined in any of claims 1 to 14 in the treatment of a  
gastrointestinal disorder.

20

16. Use according to claim 15, wherein the disorder is gastritis, peptic ulcer  
disease including both gastric and duodenal ulcers, gastric cancer, chronic  
dyspepsia with severe erosive gastroduodenitis, refractory ulcer dyspepsia,  
intestinal metaplasia, low grade MALT lymphoma, Helicobacter infection,  
25 Helicobacter pylori infection and Helicobacter felis infection.

17. A foodstuff containing a vaccine as defined in any of claims 1 to 14.

18. A foodstuff according to claim 17, in the form of a milk product.

30

19. A foodstuff according to claim 18, in the form of a yoghurt.

20. A transformed Lactobacillus spp. containing a nucleotide sequence that encodes a urease peptide capable of initiating an anti-urease cellular and/or humoral immune response upon administration to a mammalian species.

5 21. A transformed Lactobacillus according to claim 21, that is Lactobacillus plantarum.

22. A transformed Lactobacillus according to claim 20 or 21, wherein the nucleotide sequence encodes at least the ureB subunit.

10

23. A vaccine according to any of claims 1 to 14 capable of oral delivery.

# INTERNATIONAL SEARCH REPORT

Int. Application No  
PCT/GB 98/02631

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/02 C12N15/74 A61K38/43

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>EP 0 654 273 A (LEVEEN H. ET AL.) 24 May 1995</p> <p>see column 4, line 8 - column 4, line 43 see column 11, line 27 - column 12, line 40 see column 13, line 16 - column 13, line 32 see claims 28,34-36</p> <p style="text-align: center;">--- -/--</p>	<p>1-3, 10, 11, 13-16, 23</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
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- "O" document referring to an oral disclosure, use, exhibition or other means
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- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

16 December 1998

Date of mailing of the international search report

22. 01. 99

Name and mailing address of the ISA

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Authorized officer

Luzzatto, E

# INTERNATIONAL SEARCH REPORT

In International Application No  
PCT/GB 98/02631

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	POUWELS P.H. ET AL.: "The potential of Lactobacillus as a carrier for oral immunisation: development and preliminary characterisation of vector systems for targeted delivery of antigens" JOURN. BIOTECHNOL., vol. 44, 1996, pages 183-192, XP000572655 AMSTERDAM, nl see page 184, left-hand column, line 18 - left-hand column, line 41 see page 185, left-hand column, line 1 - left-hand column, line 30 see page 190, right-hand column, line 17 - page 191, left-hand column, line 26 ---	1-23
Y	WO 96 33732 A (ORAVAX, INC.) 31 October 1996 cited in the application see page 2, line 1 - page 6, line 21 see page 25, line 1 - line 14 see claims ---	1-23
A	WO 94 16086 A (BIOTEKNOLOGISK INSTITUT) 21 July 1994 see page 5, line 5 - page 8, line 24 see page 27, line 30 - line 33; claims -----	1-23

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 98/02631

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 15,16 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/GB 98/02631

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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US005843460A

**United States Patent** [19]

Labigne et al.

[11] **Patent Number:** 5,843,460[45] **Date of Patent:** Dec. 1, 1998

[54] **IMMUNOGENIC COMPOSITIONS AGAINST HELICOBACTER INFECTION, POLYPEPTIDES FOR USE IN THE COMPOSITIONS, AND NUCLEIC ACID SEQUENCES ENCODING SAID POLYPEPTIDES**

[75] **Inventors:** Agnes Labigne, Bures S/Yvette, France; Sebastin Suerbaum, Bochum, Germany; Richard L. Ferrero, Paris; Jean-Michel Thiberge, Plaisir, both of France

[73] **Assignees:** Institut Pasteur; Institut National de la Sante et de la Recherche Medicale, both of Paris, France

[21] **Appl. No.:** 467,822

[22] **Filed:** Jun. 6, 1995

**Related U.S. Application Data**

[63] Continuation of Ser. No. 447,177, May 19, 1995, which is a continuation-in-part of Ser. No. 432,697, May 2, 1995.

**[30] Foreign Application Priority Data**

May 19, 1993 [EP] European Pat. Off. .... 93 401 309  
Nov. 19, 1993 [WO] WIPO ..... PCT/EP93/03259

[51] **Int. Cl.<sup>6</sup>** ..... A61K 39/02

[52] **U.S. Cl.** ..... 424/234.1; 435/7.32; 435/6; 435/7.9; 514/234.5; 514/41

[58] **Field of Search** ..... 435/7.32, 4, 6, 435/7.9; 514/234.5, 41; 424/234.1

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(List continued on next page.)

**Primary Examiner**—James C. Housel

**Assistant Examiner**—Ginny Allen Portner

**Attorney, Agent, or Firm**—Finnegan, Henderson, Farabow, Garrett & Dunner, L.L.P.

**[57] ABSTRACT**

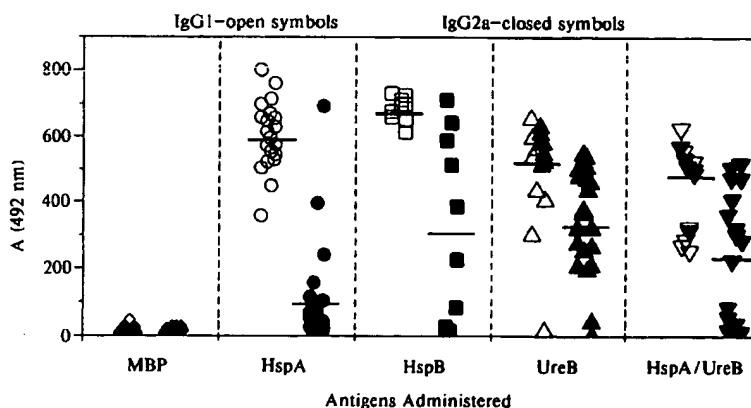
There is provided an immunogenic composition capable of inducing protective antibodies against *Helicobacter infection* characterized in that it comprises:

i) at least one sub-unit of a urease structural polypeptide from *Helicobacter pylori* (SEQ ID NOS:22,26), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter felis* urease (SEQ ID NOS:20-21), and/or at least one sub-unit of a urease structural polypeptide from *Helicobacter felis* (SEQ ID NOS:20-21), or a fragment thereof, said fragment being recognized by antibodies reacting with *Helicobacter pylori* urease (SEQ ID NOS:22-26);

ii) and/or, a heat shock protein (Hsp), or chaperonin, from *Helicobacter*, or a fragment of said protein.

The preparation, by recombinant means, of such immunogenic compositions is also provided.

10 Claims, 36 Drawing Sheets



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 C07K016/12 C07K016/40 C12N001/15 C12N001/19 C12N001/21 C12N005/10 C12N009/80  
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 G01N033/577.

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(L3 AND (UREASE OR URE OR URE-A OR URE-B).TI,AB,CLM.)).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD.	25

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□ 1: Gastroenterology. 1996 Jun;110(6):1770-5.

[Related Articles, Links](#)

ELSEVIER  
FULL-TEXT ARTICLE

Comment in:

- Gastroenterology. 1996 Jun;110(6):2003-6.

### **Therapeutic immunization against *Helicobacter mustelae* in naturally infected ferrets.**

**Cuenca R, Blanchard TG, Czinn SJ, Nedrud JG, Monath TP, Lee CK, Redline RW.**

Department of Pediatrics, Case Western Reserve University, Cleveland, Ohio.

**BACKGROUND & AIMS:** *Helicobacter* infection of the gastric antrum is responsible for a number of gastric disorders. Antibiotic therapy is lengthy and is not always effective. It has been shown previously that oral immunization against *Helicobacter felis* in mice can prevent colonization after challenge. The aim of this study was to investigate the efficacy of therapeutic immunization in eradicating an established *Helicobacter* infection and in reducing gastritis.

**METHODS:** Domestic ferrets, confirmed to be infected with *Helicobacter mustelae* by gastric endoscopy, were orally immunized with varying doses of purified *Helicobacter pylori* urease in combination with the mucosal adjuvant cholera toxin. Ferrets were assessed 1 week and 6 weeks after treatment for infection and pathology. **RESULTS:** Therapeutic immunization eradicated *Helicobacter* colonization in 30% of all immunized ferrets, although there was no difference in efficacy between the varying doses of antigen tested. The difference was statistically significant when compared with animals administered cholera toxin alone or buffer ( $P = 0.04$ ). The intensity of inflammation was also significantly reduced in immunized animals ( $P = 0.0003$ ).

**CONCLUSIONS:** Oral immunization with purified *H. pylori* urease and cholera toxin can eradicate *H. mustelae* in a natural host pathogen model. Oral immunization of chronically infected animals markedly reduced gastric inflammation.

PMID: 8964402 [PubMed - indexed for MEDLINE]

Reference molecule:	Criseq	206	2603	( 2398 bps)	Homology
Sequence 2:	Kukkasseq	48	2445	( 2398 bps)	94%
Sequence 3:	Ds4seq	2	2399	( 2398 bps)	94%
Sequence 4:	2301Seq	1	2398	( 2398 bps)	94%
Sequence 5:	390seq	3	2181	( 2179 bps)	85%
Sequence 6:	H. felis comp	43	2475	( 2433 bps)	67%
Sequence 7:	H. pylori com	2659	5088	( 2430 bps)	67%
Sequence 8:	H. helmanni	211	2636	( 2426 bps)	68%

**Alignment type:**  
**Parameters:**

Global DNA  
Mismatch 1; Open Gap 3; Extend Gap 3

Cs1seq	(	206)	gtgaactcaccacccaaagagcaagaagaatctctgttatattatctcggcggaagtgtgctagaagaagcgcaaaagcagaagggcttaagctcaaccaaccgaagccattgctcatcag
Kukka3seq	(	48)	.....
Ds4seq	(	2)	.....
2301SEQ	(	1)	.....
390seq	(	3)	.....
H. felis com	(	43)	a.....a.g.t.....a.t.....c.....aa.c.cc.....ag.t.....aga.g.aggttg.....gggt.....tg.g.a.....t.ca.....gg.c.gct.....c.....
H. pylori co	(	269)	a.....c.a.....tt.t.....ga.c.cc.c.c.t.a.t.....a.a.....a.a.a.a.t.....t.tgta.....ag.a.....tg.....
H. neilmanni	(	211)	a.....g.....t.....ttg.t.....ga.c.cc.....c.a.c.a.a.....a.a.....a.t.....a.t.....tg.a.....act.....c.....
Cs1seq	(	326)	gcccatattatgacgaagcgccgttggaaaaaaacgcttcccgctatattggaagaagtgcacgttcttgaaaaaagaatgaatgcccgggggttgtaataatgttccgcat
Kukka3seq	(	168)	.....
Ds4seq	(	122)	.....
2301SEQ	(	121)	.....
390seq	(	123)	.....
H. felis com	(	163)	gg.g.g.g.....aa.g.....tga.t.t.g.g.gg.ttg.g.c.....ag.gacaccc.....a.cc.....a.t.g.gat.c.....ca.gc.....a.c.at.a.....
H. pylori co	(	2779)	.....a.t.....a.agc.t.....g.t.cg.tg.at.g.c.....ag.gccaccc.....a.cc.....t.g.gat.c.....ca.gc.....a.c.at.a.....
H. neilmanni	(	331)	.....g.c.....a.g.c.....gcc.t.....g.....g.gg.ttg.g.c.....ag.g.act.ac.c.....gcc.....t.g.....t.c.....cgc.....a.c.a.....
Cs1seq	(	446)	ctagtgatggaagcaccacttctctgattgtctcgaagaactgtgaactgtgtaattgagcccatcgaaaccagatgagcaccctcaaacgcggcggaagtgaatttgctcgataaagacatcgag
Kukka3seq	(	288)	t.g.c.g.....t.c.c.c.....c.c.....c.....t.....a.....t.g.....t.....c.t.....t.a.....
Ds4seq	(	242)	t.....c.g.....t.t.c.c.....c.c.....c.....c.....t.....a.....t.....c.t.....t.....
2301SEQ	(	241)	t.....c.g.....t.c.c.c.....c.c.....c.....c.....t.....t.....a.....t.....c.t.....t.....
390seq	(	243)	t.g.c.t.....t.c.c.c.....c.c.....c.....c.....t.....t.....a.....t.....c.t.....t.....
H. felis com	(	283)	g.g.g.g.a.t.....t.a.c.c.c.a.c.g.....a.cc.cact.gg.a.ggata.....gca.a.agccc.c.....g.....cctaaaa.g.g.....tact.....
H. pylori co	(	2899)	g.g.g.a.t.....gtg.....g.t.....c.c.c.c.ccc.t.t.gg.ca.....gta.a.aggtt.c.t.gt.....cctaaaa.g.g.....act.....
H. neilmanni	(	451)	g.g.g.g.a.t.....ggg.....c.....g.a.....t.a.g.ca.cc.cact.g.a.a.....gt.t.gcagcg.....aagttt.tcc.....gt.aagta.cctcaaaa.cg.....acc.....
Cs1seq	(	566)	ctcaatgacgagcaagaagtaaccggaacttgaagtactactatgaaaggcgctaactcttgcatgtggtggtccattccacactcttgaagcacaagcgccataaatctgactgtgaa
Kukka3seq	(	408)	.....
Ds4seq	(	362)	.....
2301SEQ	(	361)	.....
390seq	(	363)	.....
H. felis com	(	400)	a.t.c.c.c.....cc.ttagct.g.a.a.g.aa.....a.....cga.cgtc.tg.....g.....acca.....c.....tg.t.....cctt.g.c.....c.c.c.....
H. pylori co	(	3016)	.....c.a.....a.....ccgttagcg.g.a.a.....t.....cga.g.c.gg.t.aa.c.ccca.....c.....tg.t.gatgc.....g.c.t.ca.....
H. neilmanni	(	571)	.....c.c.....c.c.ccg.t.c.t.aa.a.c.aa.....cga.cgcc.g.a.a.....tcaa.....c.....tg.t.....cctt.g.c.....c.....

**Figure 1a (1)**

## ALIGNED SEQUENCES

Reference molecule:	urexCs1	1 -	226 (	226 aa)	Homology
Sequence 2:	urexKruka	1 -	226 (	226 aa)	100%
Sequence 3:	urexD54	1 -	226 (	226 aa)	99%
Sequence 4:	urex2301	1 -	226 (	226 aa)	99%
Sequence 5:	urex390	1 -	226 (	226 aa)	50%
Sequence 6:	A felisi	1 -	237 (	237 aa)	52%
Sequence 7:	A pylori	1 -	238 (	238 aa)	52%
Sequence 8:	A hellman	1 -	234 (	234 aa)	54%

```

ureXCS1      ( 1) vltphqegkftllyyagewarkrkaegllklnqpeaiaytsahimdeartrgkktvqaqlmeecmhflkxdevmpgygmwvpdlgvaattpdqetklvtvnwplpdepdelfkagevfkfgcdkd
ureXkkuA     ( 1) .....e.....
ureXDS4      ( 1) .....e.....
ureX2301     ( 1) ?.....e.....
ureX390      ( 1) .....ld.lm.h..rl.eeal.r.v..yt.v.l.grv.ek.d.n.s.d.q.grtw.en.d.as.lhev.l.n.....ht.v.dnqrlap..lkne.t
A felis      ( 1) m.....ld.lm.h..l.k..ek.l.yv.v.l.....e.a.a.e.q.grtl.p.d.d.as.lhev.l.m.....ht.v.dnqrlap..lkne.t
A pylori     ( 1) m.....ld.lm.h..l.kg..k.l..yt.v.l.v.e..a..s.d.q.grtl.a.d..ah.lhev.l.g.....ht.v.dnqrlap..lkne.t
A hellman    ( 1) m.....ld.lm.h..l.kg..k.l..yt.v.l.v.e..a..s.d.q.grtl.a.d..ah.lhev.l.g.....ht.v.dnqrlap..lkne.t
ureXCS1      ( 121) lnagkvelevtnegpkalnhvshfnfheanakaikfdeakaygkrlidpsgntllrfgagqrkvglliplygskkvivgmnglvniaderhkhakaldakashgfl-----k
ureXkkuA     ( 121) .....e.....
ureXDS4      ( 121) .....t.....
ureX2301     ( 121) .....t.....
ureX390      ( 121) Y.....t.....sfg..a..tav.fep.eeks.e..di..n.rly.f.s.drg..adg..klg.kr..ek..gsvncgeatkdq
A felis      ( 120) l.....ais.k.k.k.drpvg.....v..l.d..a.sfg..a..tav.fep.eeks.e..di..n.rly.f.a.drg..nes.ki..hr..er..haksdndnyvtlike
A pylori     ( 120) l.e..kavsvr.k.v.drpvgl.....v..rc.d.....tf..a..tav.fep.eeks.e..di..n.rly.f.a.drg..hdg.kl..kr..eh..gtincsgcdn
A hellman    ( 121) .....havq.k.k.k.drpvg.....v..l.d.....a..tav.fep.eekt.e..di..n.rly.f.a.drg..hdg.kl..kr..eh..gtincsgcdn

```

### Figure 1b

## ALIGNED SEQUENCES

Reference molecule:	urefCS1	1 -	568 ( 568 aa)	Homology
Sequence 2:	ureyKuka	1 -	568 ( 568 aa)	99%
Sequence 3:	ureyDS4	1 -	568 ( 568 aa)	98%
Sequence 4:	ureyR301	1 -	568 ( 568 aa)	99%
Sequence 5:	urey390	1 -	496 ( 496 aa)	86%
Sequence 6:	B felis	1 -	569 ( 569 aa)	73%
Sequence 7:	B pylori	1 -	569 ( 569 aa)	73%
Sequence 8:	B hellman	1 -	568 ( 568 aa)	74%

Alignment type: Global Protein  
Parameters: Mismatch 2; Open Gap 4; Extend Gap 1; Conserv N

ureyCS1 1) mkmkkg-eyvntypgttkgdkvllgtdclwaavevndytcygeeklfkgagktliremgqsnspndclldvltcnamldtyglkadiqikngkhtgkagknkdmqdyvshmvvgvgea  
ureyXuka 1) .....t.....  
ureyDS4 1) .....t.....  
urey2301 1) .....t.....  
urey390 1) .....t.....  
B feils 1) kisk. sm. t. r. . . . . i l. . . . . C. . . . . l. g. . . . . d. s. t. . . . . ssye. l. l. v. . . . . d. a. . . . . dnlc. pa.  
B pylori 1) kisk. sm. . . . . t. . . . . l. . . . . l. . . . . g. l. . . . . s. n. skee. l. l. v. . . . . d. a. . . . . g. . . . . knlc. pa.  
B heilman 1) kisk. sm. . . . . t. . . . . t. . . . . l. . . . . C. . . . . l. g. l. . . . . d. t. . . . . sshe. . . . . l. v. . . . . knlc. pa.  
ureyCS1 120) lasegm iteagidc hthrlspqgfpccalangvtcmfgsgtgpvdgtnatittpgkwnlhzmraaeysmvgfllgkgnssakkgi vegeagaigfklihedwgttspaidhclsvade  
ureyXuka 120) .....  
ureyDS4 120) .....  
urey2301 120) .....  
urey390 120) .....  
B feils 121) ..a..l.v.. . . . . t. i. i. . . . . i. f. s. . . . . l. . . . . a. . . . . ra. ks. . . . . a. l. . . . . a. v. yeps. rd. l. . . . . i. . . . . s. a. h. n.  
B pylori 121) ..l.v.. . . . . t. i. l. i. . . . . l. f. s. . . . . l. . . . . a. . . . . re. kw. . . . . l. . . . . a. a. ndas. ad. l. . . . . l. . . . . n. a. d. k.  
B heilman 121) ..a..l.v.. . . . . t. i. l. i. . . . . l. f. s. i. l. . . . . a. . . . . r. ke. . . . . s. a. l. y. . . . . v. repa. id. l. . . . . l. . . . . s. . . . . n. a. nl. k.  
ureyCS1 240) ydqvqc hntdctvneagyvddtlnamrgxrlahybiegagsgnspdvltcmageinlpsstcptlpytlntvaeshldmmtcnhldkxiredlqfsgsrirpfsiaaedvldhdmgiants  
ureyXuka 240) .....v.....  
ureyDS4 240) .....  
urey2301 240) .....  
urey390 240) .....  
B feils 241) ..a..a.. . . . . l. . . . . C. e. . . . . e. i. a. . . . . t. t. f. t. . . . . a. . . . . k. . . . . f. . . . . a. n. . . . . f. k. e. . . . . m. . . . . v. . . . . s. k. v. . . . . ad. . . . . qt. . . . . g. . . . . q. . . . . ifsl.  
B pylori 241) ..a..a.. . . . . l. . . . . C. e. . . . . ma. i. a. . . . . tm. f. t. . . . . a. . . . . i. kv. . . . . h. . . . . a. n. . . . . f. v. e. . . . . m. . . . . v. . . . . s. k. v. . . . . ad. . . . . qt. . . . . t. . . . . ifsl.  
B heilman 241) ..a..a.. . . . . l. . . . . C. e. . . . . e. i. a. . . . . t. t. f. t. . . . . a. . . . . k. . . . . f. . . . . a. n. . . . . f. k. e. . . . . m. . . . . v. . . . . n. k. . . . . ve. ad. . . . . qt. . . . . K. . . . . ifsl.  
ureyCS1 360) sdsqamrageaviprtwtgtdknkkefklipcdgkdndmfrikryiskytlnpalthgvseiygsveegkialdvvpmpafgvykpkvlksgmvfsemgdsnasvppqpvyremg  
ureyXuka 360) .....a.....  
ureyDS4 360) .....sa.....  
urey2301 360) .....a.....  
urey390 360) .....a.....  
B feils 361) ..v..t.. . . . . t. . . . . r. k. . . . . ekg. . . . . l. . . . . g. i. a. . . . . l. d. v. . . . . v. y. . . . . l. s. . . . . l. . . . . mnd. . . . . fial. q. a. i. . . . .  
B pylori 361) ..v..t.. . . . . t. . . . . r. k. . . . . ekg. . . . . l. . . . . i. a. . . . . l. . . . . v. . . . . v. y. . . . . l. s. . . . . l. . . . . mnd. . . . . fial. q. a. i. . . . .  
B heilman 360) .....v..t.. . . . . t. . . . . r. . . . . ekg. . . . . l. . . . . i. . . . . i. . . . . v. . . . . v. y. . . . . l. s. . . . . l. . . . . mnd. . . . . fial. q. a. i. . . . .

**Figure 1c (1)**

## REVIEW

# Gastric helicobacters in cats

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The types of helicobacter which are found in the stomachs of carnivorous pets, especially cats, have been traditionally referred to as 'gastric helicobacter-like organisms' (GHLOs). These are microaerophilic, Gram-negative, spiral bacteria with multiple terminal flagellae and are endowed with high-level urease activity which allows them to survive in an acidic environment. Certain species have one or more periplasmic fibrils. The two GHLOs most commonly found in cats are *Helicobacter felis* and a species related to *H heilmannii* which was recently cultured from dogs. All phenotypic and genotypic (16S RNA gene sequences) evidence suggests that both of these bacteria belong in the genus *Helicobacter*. Whether or not helicobacters can be transmitted to humans from carnivorous pets is controversial but the recent discovery of *H pylori*-infected cats may be evidence of an animal reservoir for this pathogen. Although the role of *H pylori* in inducing antral gastritis and perpetuating pyloric ulcers in humans is well established, whether or not *Helicobacter* spp are causally involved in any feline gastric inflammatory conditions is unknown.

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Spiral bacteria in carnivorous pets were first reported by Rappin in 1881 and Bizzozero in 1893. That 'spirella' could be found in the gastric mucosae of dogs and cats was confirmed in a few subsequent studies (Salomon, 1898) but it is only recently, since the discovery by Warren and Marshall in 1983 that *Helicobacter pylori* causes disease in humans, that any kind of detailed investigation of helicobacters in carnivorous pets has been undertaken. These bacteria, which have been traditionally referred to as a 'gastric helicobacter-like organisms' (GHLOs) are mostly microaerophilic, Gram-negative, spiral species with multiple terminal flagellae and high-level urease activity which allows them to survive in an acidic environment. *Helicobacter felis* was the first species cultured from the gastric tissue of cats (Lee et al 1988, Paster et al 1991) and subsequently another common feline GHLO was identified which has not yet been cultured but which is morphologically similar to both *H heilmannii* (formerly called *Gastrospirillum hominis*) observed in some cases of human gastritis (Heilmann & Borchard 1991, Geyer et al 1993, Solnick et al 1993) and *H bizzozeronii* which was recently isolated and cultured from dogs

by Hanninen et al (1996). However, it has not been proved that *H heilmannii* and *H bizzozeronii* are the same bacterium. Finally, Handt et al (1994) have detected *H pylori* in cats and proposed that the cat might represent a significant reservoir for this bacterium, whose natural host is man.

The detection of cat GHLOs in the human stomach has indicated that they may be relatively widespread and this has led certain investigators to propose that feline helicobacters may be transmitted to humans (Wegmann et al 1991, Heilmann et al 1991, Otto et al 1994, Handt et al 1994, Fox et al 1995).

Several reports have been published on low-grade to moderate gastritis associated with GHLO infection but it has not been possible to ascribe the inflammatory lesions definitively to microbial activity (Geyer et al 1993, Otto et al 1994, Herrmanns et al 1995, Happonen et al 1996, Kanji et al 1998). Some investigations have failed to detect any correlation between gastritis and GHLO infection as both infected and uninfected cats show the same type and quantity of lesions (Geyer et al 1993, Papasouliotis et al 1997, Neiger et al 1998).

In this article, we will review the current state of knowledge concerning feline helicobacter infections.

## Morphology

Until recently, most reports describing the GHLOs of carnivorous pets have relied on purely morphological criteria (Weber et al 1958, Lockard & Boler 1970, Lee et al 1988, Geyer et al 1993) (Table 1).

Two different GHLOs have been reported in cats: '*H heilmannii*-like' and *H felis*. *Helicobacter pylori* was detected by Handt et al (1994) in cats but as this infection is apparently anecdotal, we will not describe here the morphological characters of this human bacterium.

The '*H heilmannii*-like' species is a large bacterium with a diameter of between 0.5 and 1.0  $\mu\text{m}$  and an average length of 4–8  $\mu\text{m}$ . It consists of a tightly spiralled cell with an overall rectangular shape. Its body makes a total of between four and eight turns with a gap of 0.8–1.0  $\mu\text{m}$  between each parallel strand. The bacterium's coat is smooth but at each pole there are insertion complexes from each of which project 10–20 sheathed flagellae.

*Helicobacter felis* is a medium-sized bacterium which is about 0.4  $\mu\text{m}$  thick and on average 5  $\mu\text{m}$  long (up to 7.5  $\mu\text{m}$ ). This species is also tightly spiralled with the body making between five and seven turns. Between 10 and 17 terminal sheathed flagellae are again seen at either pole but, unlike the smooth surface of the *H heilmannii*-like species, *H felis* has either one, two or four periplasmic fibrils spread out along the bacterial cell (Lee et al 1988). However two strains of *H felis* with no periplasmic fibres have been isolated (Eaton et al 1996), suggesting that this anatomical feature is not necessarily characteristic of *H felis*.

## Ecology and epidemiology

### The prevalence of GHLO infection in cats

The relationship between helicobacter and its environment (habitat, conditions for survival and development, how the infection is transmitted, etc) has been extensively studied and, in both humans and carnivorous pets, it has been shown that promiscuity, confined living conditions and poor hygiene are the most important predisposing factors for infection (Lee et al 1991, Vincent 1996, Lecoindre et al 1997a, Lecoindre et al 1998b).

The frequency of helicobacter infection in cats is high, ranging from 45 to 100% in different reports (Table 2) with the differences probably being due to differences in the detection methods used, how the animal populations were selected and also, possibly, geographical considerations. The infection rate is as high in healthy cats as in those with gastrointestinal problems (Table 2) and neither sex- nor race-dependence was observed. Some studies show a correlation between the age of an animal and the chance of its being infected (Weber et al 1958, Otto et al 1994) but more recent investigations have failed to reproduce this result (Papasouliotis et al 1997, Neiger et al 1998). Even very young infected individuals can be found—this is probably due to direct mouth-to-mouth or fecal transmission from the mother (Lee et al 1991).

Only a few studies have been conducted using methods capable of differentiating different *Helicobacter* species (eg, the polymerase chain reaction [PCR] or electron microscopy) and these suggested that the *H heilmannii*-like species was the most commonly found GHLO in the stomach, that *H felis* could be isolated less often and that the presence of *H pylori* in cats remains anecdotal (Neiger et al 1998). Infection with more than one species may occur (Lee et al 1988, Lecoindre et al 1997b).

### Location of organisms

In cats, most publications report finding bacteria in both in the gastric antrum and fundus (Geyer et al 1993, Happonen et al 1996) whereas in dogs, it was the fundus and the body of the stomach which were usually infected and bacteria were only found in other parts of the organ in the most severe cases (Lee et al 1992, Lecoindre et al 1995, Happonen et al 1996).

In cats, helicobacter organisms were found most commonly within the mucus layer but were also often observed in the crypts of fundic glands and in intracellular parietal cell canaliculi (Weber et al 1958, Herrmanns et al 1995, Happonen et al 1996). The latter focus of infection is common in carnivorous pets showing that GHLOs have an affinity for parietal cells. No such tropism is observed in human *H pylori* infection (Geyer et al 1993, Herrmanns et al 1995, Lecoindre et al 1997c, Peyrol et al 1998).

**Pathogenicity.** The pathogenicity of helicobacter has been extensively studied in recent years, mainly in animal models (Lee et al 1988, 1990,

Table 1. *Helicobacter* spp isolates in stomach of dogs, cats and humans

	<i>H pylori</i>	<i>H felis</i>	<i>H heilmannii</i>	<i>H bizzozeronii</i>	' <i>Flexispira rapini</i> '	<i>H salomonis</i>	<i>H pumetensis</i>
Cat	+	+	***				+
	(Handt et al 1994)	(Lee et al 1988)	(Jalava et al 1998)				(Neiger et al 1998)
Dog	(+)*	+	+	+	+	+	+
	(Lee et al 1990)	(Lee et al 1992)	(Jalava et al 1998)	(Hanninen et al 1996)	(Jalava et al 1998)	(Jalava et al 1997)	(Eaton et al 1996)
Human	+	(+)**	+				
	(Warren & Marshal 1983)	(Wegmann et al 1991)	(Heilmann & Borchard 1991)				

\*Experimentally established infection.

\*\*Only discovered in two cases.

\*\*\**H heilmannii*-like.

**Table 2.** Prevalence in cats in different studies

Number of cases clinically abnormal/normal	Global prevalence (%)	Prevalence in normal cat (%)	Prevalence in abnormal cat (%)	Reference
29/60	—	41	57	Geyer et al (1993)
30/24	100	100	100	Papasouliotis et al (1997)
58	91	—	—	Neiger et al (1998)
10/33	—	90	64	Kanji et al (1998)
127	76	—	—	Herrmanns et al (1995)
55	86	—	—	Otto et al (1994)
10	60	—	—	Happonen et al (1996)

Radin et al 1990). A strain's pathogenicity depends on its ability to colonise the gastric mucosa and there create an ecological niche in which it can survive avoiding the host's defence mechanisms. Different strains may also induce different levels of damage at the gastric epithelium. Motility, level of characteristic urease activity and capacity to adhere to epithelial cells are the most important factors determining the likelihood of any strain being able to establish an infection in the stomach. The organisms (which are probably taken in orally) possess an endogenous urease which allows them to catabolise urea diffusing from the mucosal surface towards the lumen of the stomach. In this way, they create a neutral micro-environment in which they can survive. As suggested by their morphological structure, these bacteria are highly motile and can migrate through the mucus to colonise the gastric epithelium. *Helicobacter pylori* which have been genetically modified to fail to produce either the urease or the flagellin monomers essential for flagella formation cannot establish infection when experimentally inoculated into animals which would normally be susceptible (Monteiro 1995). Although the great majority of the GHLOs of carnivorous pets never leave the mucus layer, where there is a pH gradient of between 2 (lumen interface) and 7 (mucosal interface), electron micrographs show that these bacteria, just like *H. pylori*, can adhere to the epithelial surface and even invade epithelial cells (Lecoindre et al 1997c, Peyrol et al 1998). This implies that adhesins are expressed on the surface of the bacterium with corresponding receptors on the mammalian cell. The unique tropism of these bacteria for the gastric epithelium is almost certainly involved in the phenomenon that infection with these bacteria persists throughout the lifetime of the infected host (Monteiro 1995). The

same colonisation factor is probably also responsible for mediating damage to the gastric mucosa.

When allowed to adhere to epithelial cells, *H. pylori* stimulated them in vitro to release interleukin-8 (IL-8) and induced structural modifications including microvillus resorption and cytoskeletal rearrangements. Similar morphological changes have been observed in vivo in both cats and dogs which supports the idea that the GHLOs of carnivorous pets may have cytotoxic activity (Lecoindre et al 1997c, Peyrol et al 1998). Bacterial phospholipase and haemolysin secretion also play a role in the pathogenesis of gastric lesions, as may the urease activity although this is not established. Certain *H. pylori* phenotypes (VacA and CagA) are more likely to cause ulcers or other more serious gastrointestinal pathologies. *Helicobacter pylori* could also play a role in the pathogenesis of autoimmune gastritis by inducing the production of antibodies specific for the Lewis antigen which is a normal component of the parietal cell  $H^+/K^+$  ATPase (a 'proton pump') (Monteiro 1995). The recent sequencing of the genomes of certain strains of *H. pylori* will almost certainly reveal other virulence genes (Labigne 1998). The idea of strain-dependent virulence is compelling in the light of the fact that, while certain helicobacter-infected carnivorous pets develop gastritis, the presence of such organisms in the stomach does not necessarily seem to cause any problem.

### GHLO infection and gastritis in cats

Several reports have been published concerning low-grade to moderate gastritis associated with GHLO infection but it has not been possible to ascribe the inflammatory lesions definitively to microbial activity (Geyer et al 1993, Otto et al 1994, Herrmanns et al 1995, Happonen et al 1996,

Kanji et al 1998). Some investigations failed to detect any correlation between gastritis and GHLO infection both clinically abnormal and normal cats showing the same type and quantity of lesions (Geyer et al 1993, Papasouliotis et al 1997, Neiger et al 1998).

The crucial issue is whether these organisms can cause disease in cats and whether any lesion can be associated with their presence. This association is not yet proven and remains unclear.

### Endoscopy

It is difficult to define a specific endoscopic pattern which is typical of GHLO infection but, if infection is severe, some or all of the following may be observed: the fundic mucosa may appear congested, oedematous and somewhat squamous; the folds of the antrum may be hypertrophic; the antral mucosa may be congested; and sometimes in adults, but especially in young subjects, elevations resembling pathological nodules may be observed (Lecoindre et al 1997b).

However, these symptoms are only ever observed in a small minority of infected animals and little importance is attached to them compared with the results from histological analysis of biopsies (Lecoindre et al 1997b, Kanji et al 1998, Neiger et al 1998).

### Pathology

Pathological analysis of gastric biopsies taken from GHLO-infected cats shows a similar picture to that seen in humans (Flejou et al 1990, Heilmann et al 1991): low grade to moderate inflammation which is usually focused around the antrum (Geyer et al 1993, Herrmanns et al 1995, Happonen et al 1996). The main inflammatory cells are lymphocytes and plasma cells, with occasional neutrophils. Multiple lymphoid follicles have been described in follicular gastritis in *H. pylori*-infected human subjects (Pariante 1993, Zerbib 1994) and similar lesions have been observed in infected cats, first by Otto et al (1994) and later by Herrmanns et al (1995), Happonen et al (1996), and Lecoindre et al (1997c). For a long time, these nodules were considered part of the normal architecture of the gastric mucosa of carnivorous pets but this is now under review in the light of results in humans which tend to indicate that this histological feature is associated with a local immune response to antigens and inflammatory mediators which are being

generated in the lumen of the stomach (probably derived from the bacteria if there is infection with helicobacter).

However, evidence of the activity of the neutrophil component of the inflammatory influx which is always present in the human disease is not a consistently observed feature in cats (Herrmanns et al 1995, Happonen et al 1996). Inflammatory lesions are often associated with early fibrosis but no correlation could be established with GHLO infection (Kanji et al 1998).

Recent electron microscopy results have confirmed not only that feline helicobacter organisms bind efficiently to host cells but also that they can gain intracellular access. Intracellular GHLOs are associated with signs of cellular degeneration including mitochondrial swelling, the formation of cytoplasmic vacuoles around the bacteria and the blebbing of organelles (Herrmanns et al 1995, Lecoindre et al 1997c). Peyrol et al (1998) showed that, in dogs, only *H. felis* induced this kind of lesion and that heavy *H. heilmannii* infection was not associated with damage at the gastric epithelium. This observation has not been confirmed in cats in which it seems that the combination of different GHLOs proves the most pathogenic (Lecoindre et al 1997c).

### The clinical picture

The main symptoms of GHLO-associated gastritis in cats are chronic vomiting and diarrhoea (Geyer et al 1993, Herrmanns et al 1995), while weight loss, dysorexia and fever occur less often. In some cases haematemesis or melaena and anaemia may be observed if there is concurrent erosive or ulcer disease (Fox 1995). Regression after treatment with antacids or antisecretories appears complete and rapid. In addition, many GHLO infections are completely asymptomatic (Happonen et al 1996, Papasouliotis et al 1997, Neiger et al 1998).

The relationships between GHLOs and clinical manifestations are unclear because GHLOs have been found in clinically normal and abnormal cats and the prevalence of GHLO infection in cats is not higher than that in those which were clinically normal (Kanji et al 1998).

### Diagnosis of helicobacter infection

There are many different methods of diagnosing helicobacter infection in humans and most of

these can be adapted for animals. A distinction is made between direct, or invasive, tests (which require endoscopically obtained gastric tissue samples) and indirect, non-invasive, tests which are of particular value for high volume epidemiological investigations and in post-treatment follow-up.

### Direct methods

All direct methods initially entail peroral endoscopy in order to obtain biopsy material. GHLOs seem to be localised in all regions of the stomach in cats (Happonen et al 1996). This may mean that only samples from the fundus and corpus are sufficient to demonstrate the presence of GHLOs.

**Rapid urease test.** This procedure involves incubation of tissue samples in a urea broth containing a pH indicator (phenol red). GHLOs have high-level urease activity that breaks down urea into ammonia. This raises the pH, thereby causing an indicator to change colour. This test can be used for rapid detection of helicobacter infection and can even be performed before the endoscope has been withdrawn. The speed at which the indicator changes colour can be used to estimate the bacterial load (Otto et al 1994, Kanji et al 1998). Because at least 104 organisms are required for the rapid urease test to produce a positive result, the sensitivity of the test is only 70–90% (Jenkins 1997, Neiger et al 1998).

**Histopathological examination.** GHLOs can be visualised in sections stained with haematoxylin and eosin, haematoxylin-phloxine/safranin, Gram or Warthin-Starry silver. This test has high sensitivity and specificity, but false-negative results are possible because the distribution of bacteria in gastric tissue is often patchy. Analysis of multiple biopsies from the corpus, fundus and antrum is recommended.

This test is usually used as the reference method for studies in animals.

**Brush cytology.** This direct technique can easily be performed with a cytology brush introduced into the operating canal of the endoscope and is currently in use.

**Culture.** All *Helicobacter* species are micro-aerophilic and require special incubation conditions (5% oxygen, 10% carbon dioxide and 85% nitrogen) because the bacteria die at an oxygen

tension of 20% at normal atmospheric pressure. For this reason, the culture of helicobacter is not a routine procedure. *Helicobacter felis* proved the easiest to isolate but some *Helicobacter* species have not yet been successfully cultured (Jalava et al 1998).

**PCR.** Certain species-specific DNA sequences lend themselves to amplification-based techniques, eg, different feline GHLOs have different urease genes (ureA and ureB) (Handt et al 1994, Jalava et al 1998, Neiger et al 1998) which can be differentiated by analysing PCR products thereby showing which kind of bacterium and which species are present in the sample. Unfortunately, recent data show that the levels of 16S rDNA sequence similarity between *H felis*, *H bizzozeronii* and *H heilmannii* are extremely high, and this widely used gene does not seem suitable as a target for species-specific PCR (Jalava et al 1997).

### Indirect methods

**[<sup>13</sup>C]-urea respiratory test.** The [<sup>13</sup>C]-urea respiratory test is an indirect diagnostic test based on the high level of urease activity in *Helicobacter*. The test was known to be both sensitive and specific for diagnosing human *H pylori* infections, has been shown to have sensitivity and specificity levels of over 95% in dogs (Ballèvre et al 1997) and has recently been validated for use in cats with its results correlating closely with those of other methods (Neiger et al 1998). The maximum change in <sup>13</sup>C over baseline values was found 30 min post-dosing. This test is particularly useful for post-treatment follow-up and for high-volume epidemiological investigations.

**Serology.** Serum samples can be evaluated by kinetic enzyme-linked immunosorbent assay (ELISA) in the detection of the presence of *H felis*. High-molecular-weight cell-associated protein, purified from a detergent extraction of *H felis* ATCC 49179 has been used in a recent study (Simpson et al 1999).

## Treating GHLO infections

The discovery of the pathogenicity of *H pylori* in humans led to the development of treatment strategies designed to eliminate the bacterium. The first therapeutic regimes were based on combinations of bismuth salts with one or two antibiotics, but nowadays triple antibiotic

therapy is considered the most effective way of sterilising the bacterium. The concomitant prescription of antisecretories (especially proton pump inhibitors) is necessary to guarantee antibiotic activity which would otherwise be inhibited by the low pH. The combination of antimicrobial drugs with different sites of action (eg, local together with systemic) not only reduces the treatment time (to 7–14 days) and the amounts of drugs needed but also reduces the risk of the development of drug resistance. Various different combinations have been approved in humans, mostly based on an imidazole antibiotic (metronidazole or tinidazole) together with a macrolide (erythromycin or clarithromycin) and amoxicillin (Vincent 1996).

Because GHLO infection is so prevalent in animals, yet no pathogenic role has been defined, the decision whether or not to treat the infection is a difficult one. In our opinion, various different considerations should be taken into account including whether or not there are any problems in the upper digestive tract, histological evidence of moderate to severe gastritis and confirmation of high numbers of infecting GHLOs.

Few controlled studies of the treatment of GHLO infection have been performed in carnivorous pets. Most veterinarians have directly adapted the kind of treatment regime used against *H pylori* in humans (Vincent 1994, Novo et al 1995, Lecoindre et al 1998a). We use the combination of metronidazole (methyl-nitroimidazol-ethanol group) 30 mg/kg/day, spiramycin (erythromycin group) 15000 iu/5 kg/day and omeprazole (proton group inhibitor) 1 mg/kg/day, over 7 days. The results of this study in dogs indicate that this treatment is effective (clearance 100%, eradication 80% 40 days after treatment) and without side-effects (Lecoindre et al 1998b). The regression of chronic fundic gastritis was 50%, but these results could not be proved in cats.

In brief, the important questions regarding the treatment of GHLO infection in cats that justify further investigation are: the appropriateness of treating GHLO infection; the eradication rate following treatment; and the reinfection rates after treatment.

### Can GHLOs be transmitted from cats to humans?

The zoonotic transmission of gastric helicobacter organisms is the subject of much active discus-

sion and many alternative hypotheses, but it is true to say that spiral bacteria resembling feline helicobacter ('*H heilmannii*-like' and *H felis*) have been found in gastritic lesions in the stomachs of human subjects (Flejou et al 1990, Heilmann et al 1991). A recent report suggests that '*H heilmannii*' infection is an example of zoonosis. The authors conclude that human and animal *H heilmannii* strains are closely related and that the humans can be infected by more than one *H heilmannii* strain, as has been observed for *H pylori* (Dietrich et al 1998).

More recently, the detection of *H pylori* in cats in the same niche could indicate that cats could act as a reservoir for the bacterium (Handt et al 1994). Since other work has shown that the saliva, feces and gastric juice of *H pylori*-infected cats can contain viable pathogens (Fox et al 1995) and given that cats are continuously licking their fur, that they vomit frequently and that humans regularly come into contact with their feces when changing their litter, the presence of *H pylori* in these feline excretions means that transmission to a human host may be possible. However, the actual frequency of *H pylori* infection in cats has not yet been properly determined and could be minimal because, on present evidence, it seems that cats in their natural environment (not raised in contact with humans) are rarely infected (Eaton et al 1996).

### Conclusions

In the light of the results from a wide variety of studies, it is clear that GHLOs infect a large proportion of all cats and that, in certain conditions, these bacteria can induce a local mucosal inflammatory response which is probably chronic. Just like *H pylori*, these bacteria possess certain features, such as high urease activity and motility, which confer on them the ability to establish infection in the gastric mucosa. However, many questions still need answering. What role do these bacteria play in the pathogenesis of inflammatory gastric conditions in cats? Are some species more pathogenic than others? Are combinations of different species more damaging to the gastric epithelium?

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## Effect of Oral Immunization with Recombinant Urease on Murine *Helicobacter felis* Gastritis

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The ability of oral immunization to interfere with the establishment of infection with *Helicobacter felis* was examined. Groups of Swiss Webster mice were immunized orally with 250 µg of *Helicobacter pylori* recombinant urease (rUrease) and 10 µg of cholera toxin (CT) adjuvant, 1 mg of *H. felis* sonicate antigens and CT, or phosphate-buffered saline (PBS) and CT. Oral immunization with rUrease resulted in markedly elevated serum immunoglobulin G (IgG), serum IgA, and intestinal IgA antibody responses. Challenge with live *H. felis* further stimulated the urease-specific intestinal IgA and serum IgG and IgA antibody levels in mice previously immunized with rUrease but activated primarily the serum IgG compartment of PBS-treated and *H. felis*-immunized mice. Intestinal IgA and serum IgG and IgA anti-urease antibody responses were highest in rUrease-immunized mice at the termination of the experiment. Mice immunized with rUrease were significantly protected ( $P \leq 0.0476$ ) against infection when challenged with *H. felis* 2 or 6 weeks post-oral immunization in comparison with PBS-treated mice. Whereas *H. felis*-infected mice displayed multifocal gastric mucosal lymphoid follicles consisting of CD45R<sup>+</sup> B cells surrounded by clusters of Thy1.2<sup>+</sup> T cells, gastric tissue from rUrease-immunized mice contained few CD45R<sup>+</sup> B cells and infrequent mucosal follicles. These observations show that oral immunization with rUrease confers protection against *H. felis* infection and suggest that gastric tissue may function as an effector organ of the mucosal immune system which reflects the extent of local antigenic stimulation.

Infection with *Helicobacter pylori* is associated with the development of gastritis, peptic ulceration, and gastric carcinoma (1, 27). Gastric tissues infected with *Helicobacter* spp. harbor lymphoid follicles (12, 32) driven by local antigen stimulation (40). Populations of CD3<sup>+</sup>, CD4<sup>+</sup>, and αβ T-cell receptor (αβTCR)-positive T cells recruited into gastric mucosa may regulate B-cell function and local immunoglobulin A (IgA) antibody secretion (12, 35). While *H. pylori* antigens activate peripheral blood T cells and B cells in vitro (20), the antigen specificity of lymphocytes resident in gastric mucosae has not been examined directly. Serum IgG and local mucosal IgA antibody responses are developed in response to infection with *Helicobacter* spp., although these appear to be insufficient for clearance (5, 12, 30, 37).

The urease molecule from *H. pylori* is an essential determinant of pathogenicity. Because urease-negative mutants fail to colonize gastric tissue of gnotobiotic piglets (8) and nude mice (36), urease may represent an important target for immunization and prevention of disease. Urease is a multimeric enzyme composed of two structural subunits of 29.5 (UreA) and 66 (UreB) kDa (7, 18) and is localized in both the cytoplasm and on the surface of *H. pylori* (15). The *H. pylori* urease molecule exhibits a high degree of sequence conservation in comparison with that of *Helicobacter felis* urease (11).

A model of chronic persistent gastritis using mice infected with *H. felis* has allowed the study of immune responses and disease progression in chronically infected animals (12, 23). Using this model, recent studies have shown that oral immunization with *H. felis* antigens results in protection against subsequent challenge with *H. felis* organisms (2, 6, 22). While

mucosal immunization strategies which prevent *Helicobacter* infection generate mucosal IgA and serum IgG and IgA antibodies (2, 6), parenteral immunization yielding high levels of serum IgG does not confer protection against challenge (3, 9). The experiments presented herein examined the ability of orally administered recombinant urease (rUrease) antigen to protect mice from *H. felis* infection and *H. felis*-associated gastritis. We show the mucosal IgA and serum IgG and IgA antibody responses in immunized mice subsequently challenged with live *H. felis* and the gastric lymphocyte cytoarchitecture in infected and protected mice.

### MATERIALS AND METHODS

**Animals.** Twenty-eight germfree 4-week-old female Swiss Webster mice were obtained from Taconic Farms (Germantown, N.Y.). The mice were maintained in a germfree isolator and subsequently housed in barrier conditions (12) for the duration of the experimental treatments. All materials for the germfree unit were sterilized by peracetic acid, and the mice were fed an autoclaved pelleted diet and given sterile water ad libitum.

**Bacteria.** *H. pylori* ATCC 43505 was cultured on Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood and 10 µg of vancomycin per ml, 10 µg of trimethoprim per ml, and 10 µg of polymyxin B (Sigma Chemical Co., St. Louis, Mo.) per ml. *Escherichia coli* BL21-DE3 (Novagen, Madison, Wis.) harboring plasmid pORV154 was cultured in liquid medium in Luria broth (Difco Laboratories, Detroit, Mich.) containing 50 µg of ampicillin (Sigma) per ml or on Luria broth plates containing 1.5% agar and 100 µg of ampicillin per ml. The *H. felis* ATCC 49179 used for oral challenge was grown under microaerobic conditions on 5% lysed horse blood agar supplemented with 10 µg of vancomycin per ml, 5 µg of trimethoprim lactate per ml, 3 µg of polymyxin B (Sigma) per ml, and 2.5 µg of amphotericin per ml as described elsewhere (23). The bacteria were harvested, inoculated in brain heart infusion agar with 30% glycerol, and frozen at -70°C. Prior to use, aliquots were thawed, analyzed for motility, and cultured for evidence of aerobic or anaerobic bacterial contamination.

**Preparation of *H. felis* antigens and native *H. pylori* urease.** *H. felis* sonicate antigens were prepared as described elsewhere (13). In brief, *H. felis* was grown for 48 h in brucella broth (Difco) containing 5% fetal calf serum. Cultures were incubated at 37°C in a microaerobic environment and shaken at 120 rpm. The cultures were centrifuged at 10,000 rpm (Sorvall RC-5B, Newtown, Conn.) for 10 min, the pellet was washed in phosphate-buffered saline (PBS), and the cells were disrupted by sonication (Artec K System, Inc., Farmingdale, N.Y.). After

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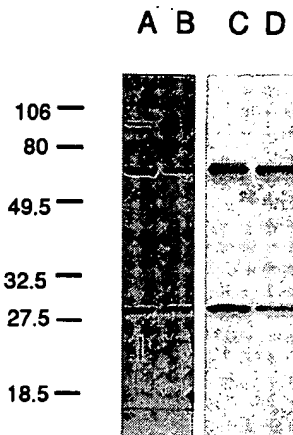


FIG. 1. Characterization of *H. pylori* native urease and rUrease. Purified urease (5  $\mu$ g) derived from *E. coli* containing the UreA and UreB genes of *H. pylori* (lanes A and C) or from *H. pylori* ATCC 43505 (lanes B and D) was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels (lanes A and B) or transferred to nitrocellulose for Western blotting (lanes C and D) with mouse polyclonal antibody raised against *H. pylori* apoenzyme. Molecular size standards are shown in kilodaltons.

centrifugation at 10,000 rpm (Sorvall RC-5B; Dupont) for 10 min, the protein content was determined (25) and aliquots were frozen at  $-70^{\circ}\text{C}$  until used. Urease from *H. pylori* was purified from organisms harvested from blood agar plates. The *H. pylori* organisms were lysed, centrifuged, and subjected to chromatography on DEAE-Sepharose (Pharmacia, Piscataway, N.J.) (18). The bound material was eluted with 150 mM NaCl, concentrated, and applied to a Sephacryl 300 sizing column (Pharmacia). Eluted fractions were assayed for the presence of urease activity (16). Fractions containing urease activity were then bound to Mono-Q-Sepharose (Pharmacia) and eluted with a 0 to 1 M NaCl gradient. The fractions containing urease activity were pooled, concentrated, and stored in 50% glycerol at  $-20^{\circ}\text{C}$ .

**Cloning and purification of *H. pylori* rUrease.** *H. pylori* rUrease was derived from *E. coli* ORV154 expressing the structural genes for the A (UreA) and B (UreB) subunits (4, 21) required for assembly (17). ORV154 was constructed as described elsewhere (24). rUrease was expressed constitutively from the T7 promoter (33) of pGEM3Z and purified as follows. ORV154 was cultured overnight at  $37^{\circ}\text{C}$  in shake flasks. The organisms were harvested by centrifugation, washed in PBS, and lysed by sonication. Contaminating proteins were removed from cell extracts by chromatography on DEAE-Sepharose (Pharmacia) and then by a 50 mM NaCl wash. The eluate was diluted 10- to 20-fold and then bound to DEAE-Sepharose. Urease was eluted with 150 mM NaCl, and fractions containing urease were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting (immunoblotting) (34) using a mouse polyclonal antibody raised against *H. pylori* urease. The urease-containing fractions were pooled, concentrated, and subjected to Sephacryl 300 sizing chromatography. This procedure yielded enzymatically inactive rUrease exhibiting two major bands of 60 and 30 kDa, corresponding to the B and A subunits, respectively (Fig. 1), with >90% purity, as assessed by scanning densitometry (Pharmacia-LKB Ultrosan).

**Experimental protocol for oral immunization.** Mice were divided into three groups and immunized per os on days 0, 10, 20 and 30 with a blunt feeding needle (Popper & Sons, Inc., New Hyde Park, N.Y.) as follows: group 1, PBS and 10  $\mu$ g of cholera toxin (CT; Calbiochem, La Jolla, Calif.;  $n = 8$ ); group 2, 1 mg of *H. felis* sonicate and 10  $\mu$ g of CT ( $n = 10$ ); and group 3, 250  $\mu$ g of *H. pylori* rUrease plus 10  $\mu$ g of CT ( $n = 10$ ). Groups of mice were challenged with live *H. felis* three times at 2-day intervals (12) either 2 weeks (day 45) or 6 weeks (day 73) after the last oral immunization.

**Culture for *H. felis* and urease tests.** From 31 to 32 days (2-week postimmunization groups) or 41 to 42 days (6-week postimmunization groups) post-*H. felis* challenge, the mice were euthanized with an overdose of carbon dioxide. Two-millimeter cubes of gastric mucosa from the antrum, fundus, and duodenum were collected aseptically for culture or for the tissue urease test (12, 16).

**Determination of antibody levels to urease and *H. felis* antigens in serum and feces.** Blood was obtained from the retro-orbital sinus 7 days after the second and fourth immunizations, 13 to 14 days after *H. felis* challenge, and by cardiac puncture at the termination of the experiment. Secretory IgA was extracted from fecal pellets (14) by incubation in PBS containing 5% nonfat dry milk, 0.2 mM 4-(2-aminocetyl)-benzenesulfonylfluoride (Calbiochem), 1  $\mu$ g of aprotinin per ml, 10  $\mu$ M leupeptin (Sigma), and 3.25  $\mu$ M bestatin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.). After extensive vortexing, the fecal material

TABLE 1. Effect of oral immunization on the presence of *H. felis* in gastric tissue<sup>a</sup>

Treatment group	Time postimmunization of <i>H. felis</i> challenge (wk)	No. of mice positive/total no. by:		
		Urease test	Culture	Warthin-Starry histology
PBS	2	3/4	4/4	3/4
<i>H. felis</i> sonicate	2	0/5 <sup>b</sup>	1/5 <sup>c</sup>	0/5 <sup>b</sup>
<i>H. pylori</i> rUrease	2	0/5 <sup>b</sup>	0/5 <sup>d</sup>	0/5 <sup>b</sup>
PBS	6	1/4	3/4	2/4
<i>H. felis</i> sonicate	6	0/5	0/5 <sup>b</sup>	0/5
<i>H. pylori</i> rUrease	6	0/5	0/5 <sup>b</sup>	0/5

<sup>a</sup> Groups of germfree Swiss Webster mice were orally immunized and challenged per os three times at 2-day intervals with  $10^8$  *H. felis* organisms. After 31 to 32 days (2-week postimmunization groups) or 41 to 42 days (6-week postimmunization groups), gastric tissue was examined for the presence or absence of *H. felis* infection as indicated.

<sup>b</sup>  $P = 0.0476$  by Fisher's exact test compared with value of corresponding PBS group.

<sup>c</sup>  $P = 0.0238$  by Fisher's exact test compared with value of corresponding PBS group.

<sup>d</sup>  $P = 0.0079$  by Fisher's exact test compared with value of corresponding PBS group.

was centrifuged (16,000  $\times g$  for 10 min), and the supernatants were used for determination of antibody. An enzyme-linked immunosorbent assay (ELISA) was used for antibody measurement. In brief, triplicate wells of microtiter plates (Dynatech, Chantilly, Va.) were incubated with purified *H. pylori* urease or with *H. felis* sonicate preparations (100  $\mu$ g/ml) in carbonate buffer. After washing with PBS-0.5% Tween 20, the wells were blocked with PBS-Tween containing 2.5% nonfat dry milk and incubated for 1 h at  $37^{\circ}\text{C}$  with serial dilutions of sera or fecal extracts. The wells were then incubated with biotinylated goat-anti mouse IgG or goat-anti-mouse IgA (Southern Biotechnology, Birmingham, Ala.) and then with streptavidin-alkaline phosphatase (Calbiochem). Negative control sera and fecal extracts and positive serum controls with known anti-*H. felis* activity were included in each assay.

**Histopathology.** Longitudinal sections of gastric tissue, from the esophageal junction through the duodenum, were fixed in 10% neutral buffered formalin. Stomachs were processed for routine histology and embedded in paraffin, and 5- $\mu$ m sections were stained with hematoxylin and eosin. Sections of the fundus, antrum, and duodenal-pyloric junction were examined in a coded fashion for histological changes and for the presence of *H. felis* in Warthin-Starry-stained specimens.

**Immunohistochemistry.** Longitudinal sections of gastric tissue including the corpus and antrum were mounted in O.C.T. compound (Miles Scientific, Naperville, Ill.) and frozen in liquid-nitrogen-cooled Freon 22 (12). Tissue sections (7  $\mu$ m) were fixed with acetone, and biotin-avidin-binding sites were blocked for 30 min (Vector Laboratories, Burlingame, Calif.). Tissue sections were incubated with biotinylated monoclonal antibodies (MAb; see below) and then with avidin conjugated to biotinylated horseradish peroxidase (ABC; Vector Laboratories) as described elsewhere (12). Controls included incubation with a MAb of unrelated specificity. Cell-bound peroxidase was visualized with 0.05% diaminobenzidine tetrahydrochloride (Organon Teknica, Durham, N.C.) and 0.01%  $\text{H}_2\text{O}_2$  in PBS, and sections were counterstained with methyl green. Baseline values of infiltrating leukocytes were established with groups of known PBS-treated and challenged mice. The tissue sections from the remaining experimental groups for immunohistology were scored by a code. The degree of gastric infiltration and/or expression of antigens defined by the MAb was scored as mild (+), moderate (++), or severe (+++).

**MAb.** The following MAb recognizing lymphocyte cell surface structures were used in this study: anti-Thy1.2 (clone 30-H12), anti-CD4 (clone GK1.5), anti-CD8 (clone 53-6.7) (Becton Dickinson, San Jose, Calif.), anti-CD3 (clone 145-2C11), anti-CD5 (clone 53-7.3), anti-CD49d (VLA-4; clone MFR 4.B), anti-CD62L (L-selectin; clone MEL-14), anti- $\alpha\beta$ TCR (clone H57-597), anti- $\gamma\delta$ TCR (clone GL3), anti-I-A<sup>d</sup> (clone AMS-32.1), and anti-I-A<sup>p</sup> (clone 7-16.17) (Pharmingen, San Diego, Calif.). The MAb anti-CD45R (B220; clone RA3-3A1/6.1), anti-IgM (clone 331.12), and anti-CD11b (M1/70.15) were obtained from the American Type Culture Collection (Rockville, Md.). The MAb 10-4.22 was used to identify IgA-positive B cells (13, 31).

## RESULTS

**Effect of oral immunization on *H. felis* infection.** The outcome of gastric urease tests, bacterial cultures, and histological identification of *H. felis* organisms by Warthin-Starry stain of

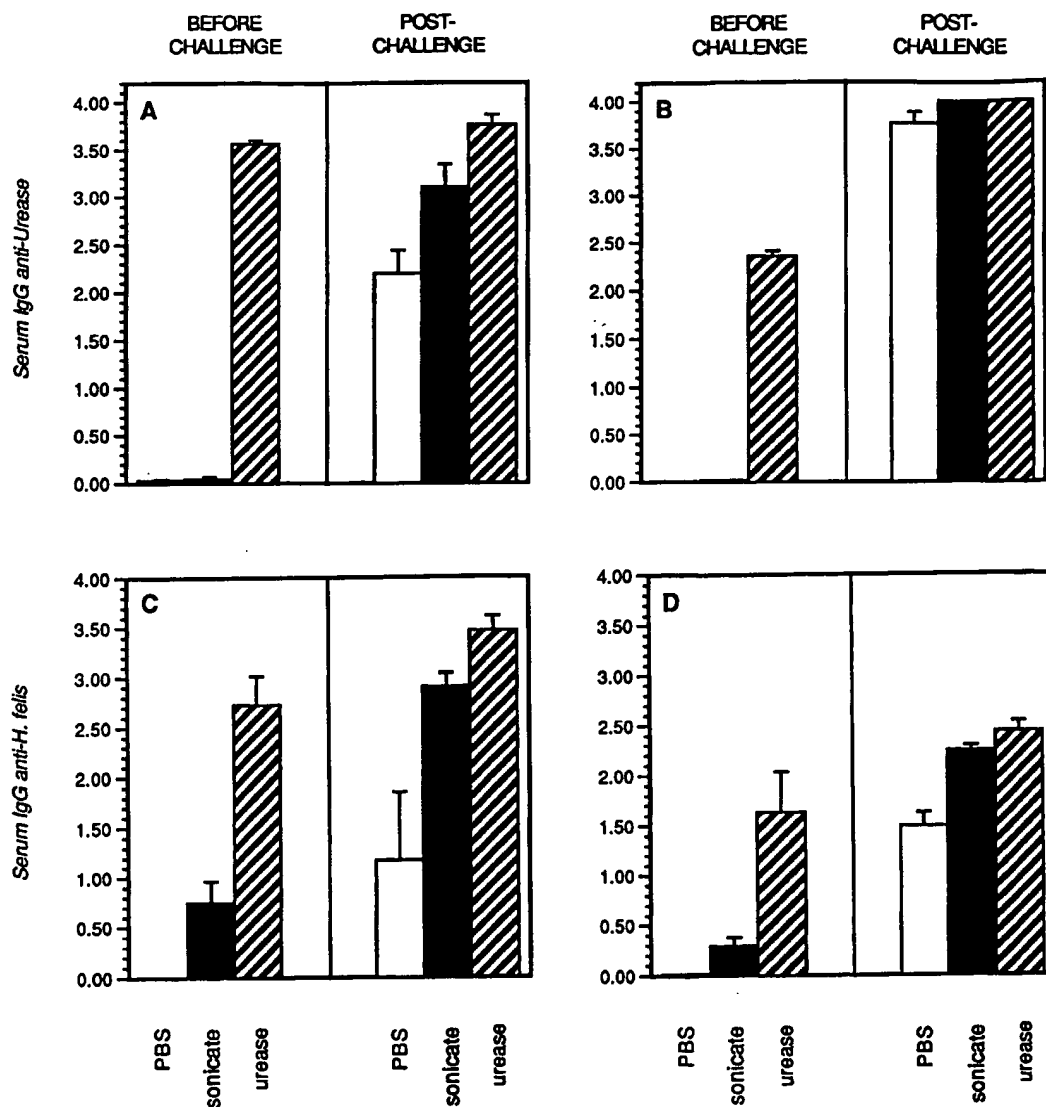


FIG. 2. Antibody responses in sera of immunized mice. Groups of Swiss Webster mice were immunized orally with PBS and 10  $\mu$ g of CT (open bars;  $n = 4$ ), 1 mg of *H. felis* sonicate and 10  $\mu$ g of CT (filled bars;  $n = 5$ ), or 250  $\mu$ g of *H. pylori* rUrease and 10  $\mu$ g of CT (striped bars;  $n = 5$ ). The mice were challenged with live *H. felis* 2 weeks (A and C) or 6 weeks (B and D) later, and serum IgG anti-urease antibody (A and B) or serum IgG anti-*H. felis* antibody (C and D) levels were quantitated by ELISA. Values from immunized animals before challenge with *H. felis* were derived from samples taken 7 days after the last immunization. Values from postchallenge mice were obtained by analysis of samples harvested 13 to 14 days after *H. felis* challenge. The bars show the mean optical density at 405 nm for each group, and the brackets enclose 1 standard error of the mean.

gastric tissue is shown in Table 1. Immunization with *H. pylori* rUrease or with *H. felis* sonicate antigens interfered ( $P \leq 0.0476$ ) with the establishment of infection upon challenge with live *H. felis* 2 or 6 weeks after the last immunization dose. The gastric tissues of PBS-treated mice challenged with *H. felis* remained colonized during the time periods examined.

**Serum antibody levels to *H. pylori* urease and *H. felis* antigens in orally immunized mice.** Oral immunization with rUrease resulted in the development of urease-specific serum IgG in 9 of 10 mice after two immunizations and in marked elevation of serum IgG anti-urease antibody in 10 of 10 mice following four immunizations (Fig. 2A and B). Serum IgA antibody was assayed consistently in rUrease-immunized mice only after the fourth oral immunization. Specific antibody responses

directed against urease were not measurable in PBS-treated mice nor in mice immunized with *H. felis* sonicate. Oral immunization with rUrease also elicited a greater serum IgG antibody against *H. felis* sonicate antigens than that generated in mice immunized with *H. felis* sonicate (Fig. 2C and D). Challenge of mice with *H. felis* either 2 or 6 weeks postimmunization further stimulated the serum IgG anti-urease antibody response of rUrease-immunized mice and activated the serum IgG antibody compartment of PBS-treated and *H. felis*-immunized animals. Whereas *H. felis* challenge similarly stimulated the urease-specific serum IgA of rUrease-immunized mice, these levels constituted about 40% of the serum IgG antibody level (not shown). The highest level of serum IgA antibody against *H. felis* was measured in mice challenged 6 weeks post-

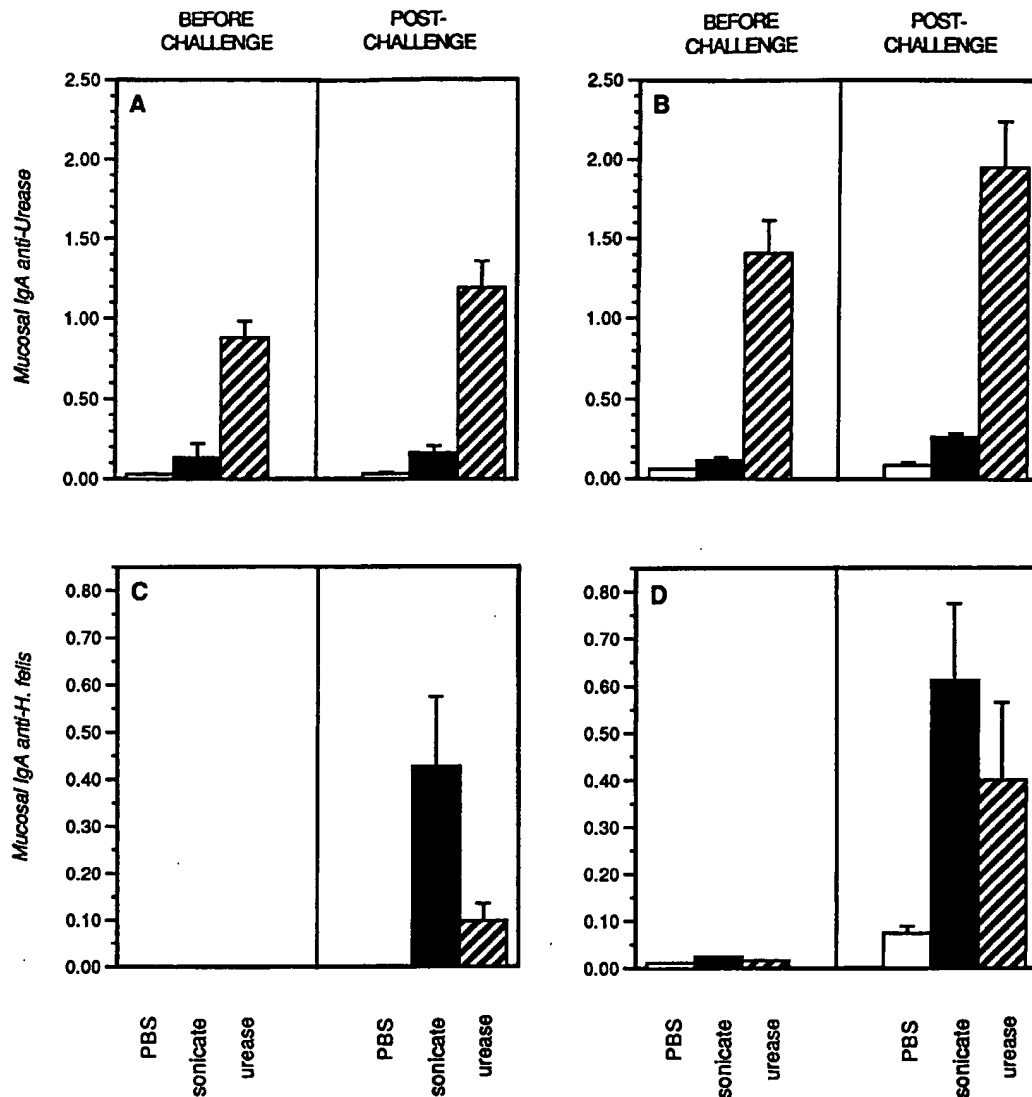


FIG. 3. Secretory IgA antibody levels in the intestinal compartment of Swiss Webster mice immunized orally with PBS (open bars;  $n = 4$ ), *H. felis* sonicate (filled bars;  $n = 5$ ), or *H. pylori* rUrease (striped bars;  $n = 5$ ). The mice were challenged with live *H. felis* 2 weeks (A and C) or 6 weeks (B and D) after the last immunization, and the fecal IgA antibody levels against urease (A and B) or *H. felis* (C and D) were quantitated by ELISA. The bars show the mean optical density at 405 nm for each group, and the brackets enclose 1 standard error of the mean.

rUrease immunization (optical density at 405 nm =  $0.337 \pm 0.117$ ). The magnitude of both serum IgG and IgA antibody responses against rUrease and *H. felis* antigens at the termination of the experiment was greatest in mice immunized with rUrease.

**Intestinal antibody responses.** The specific fecal IgA antibody response against urease and *H. felis* antigens before and after *H. felis* challenge is shown in Fig. 3. Oral immunization with rUrease generated much greater IgA anti-urease antibody levels than did immunization with *H. felis* sonicate antigens. Whereas all mice immunized with rUrease developed anti-urease IgA, fecal urease-specific antibody responses were low (optical density at 405 nm < 0.16) or were not measurable in groups of mice immunized with *H. felis* sonicate. Challenge of rUrease-immunized mice with *H. felis* 2 or 6 weeks postimmunization increased the urease-specific intestinal IgA antibody

level. However, *H. felis* challenge of PBS-treated control mice or *H. felis* sonicate-immunized mice had little effect on the intestinal IgA anti-urease antibody concentration (Fig. 3A and B). Although the fecal IgA antibody levels against *H. felis* resulting from oral delivery of antigen were very low, oral immunization of mice with rUrease or with *H. felis* antigens effectively primed the mucosal IgA compartment, as shown by the generation of much greater IgA responses in antigen-immunized animals after inoculation with live *H. felis* (Fig. 3).

**Histopathology and immunohistopathology of gastric tissue.** Inflammatory changes were observed in all mice in both the body and the antral regions in the subglandular portion of the mucosa and frequently extended into the underlying submucosa. The cell infiltrates were characterized as active chronic inflammation and were present as multifocal, relatively diffuse aggregates of mononuclear leukocytes and neutrophils.

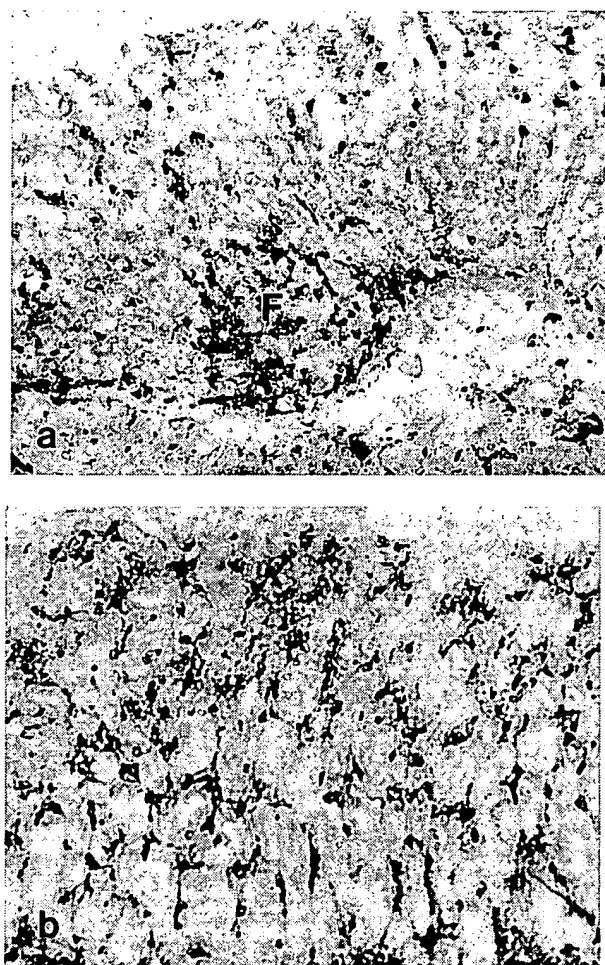


FIG. 4. Immunohistochemical localization of infiltrating Thy1.2<sup>+</sup> T cells in cross sections of gastric mucosa approximately 40 days post-oral inoculation with *H. felis*. (a) Gastric tissue from *H. felis*-infected mice immunized with PBS showing lymphoid follicles (F) delimited by scattered mucosal Thy1.2<sup>+</sup> T cells. (b) The gastric mucosa from rUrease-immunized mice subsequently challenged with *H. felis* exhibiting predominant clusters of mucosal and submucosal Thy1.2<sup>+</sup> T cells. Magnification,  $\times 500$ .

Immunohistochemical analyses of PBS-treated control animals challenged with *H. felis* revealed multifocal CD45R<sup>+</sup> and IgM<sup>+</sup> B cells assembled into mucosal and submucosal lymphoid follicles frequently surrounded by aggregates of Thy1.2<sup>+</sup> T cells (Fig. 4). In contrast, the gastric tissue from rUrease- or *H. felis* sonicate-immunized animals contained scattered CD45R<sup>+</sup> B cells and occasional mucosal follicles but exhibited mild to moderate infiltration by Thy1.2<sup>+</sup> T cells distributed as single cells or as clusters in the gastric mucosa and submucosa (Fig. 4 and Table 2). All mice showed a similar distribution of CD3<sup>+</sup> CD4<sup>+</sup>  $\alpha\beta$ TCR-positive T cells. CD8<sup>+</sup> or  $\gamma\delta$  T cells were infrequently observed in gastric mucosae. Populations of CD5<sup>+</sup>, CD11b<sup>+</sup>, and IgA<sup>+</sup> cells occurred as isolated cells or as small clusters of cells in the gastric pits, lamina propria, and epithelium. The gastric epithelium and infiltrating mononuclear leukocytes were substantially I-A<sup>P</sup> (Table 2). The architectural compositions of cell populations infiltrating gastric tissue were similar whether mice were challenged with *H. felis* 2 or 6 weeks after the last oral immunization.

TABLE 2. Relative phenotypic distributions of mononuclear leukocytes infiltrating gastric tissue after oral immunization and challenge with *H. felis*

Antigen structure or CD designation	Findings for experimental group immunized with <sup>a</sup> :		
	PBS	<i>H. felis</i> sonicate	rUrease
Thy1.2	+	+	+
CD3	+	+	+
CD4	+	+	+
CD5	+	+	+
CD8	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
$\alpha\beta$ TCR	+	+	+
$\gamma\delta$ TCR	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
CD45R	+++	+	+
IgA	+ <sup>c</sup>	+ <sup>d</sup>	+ <sup>d</sup>
IgM	++ <sup>e</sup>	+ <sup>d</sup>	+ <sup>d</sup>
CD11b	+	+	+
CD49d (VLA-4)	+	+	+
CD62L (L-selectin)	— <sup>b</sup>	— <sup>b</sup>	— <sup>b</sup>
I-A <sup>P</sup>	+++ <sup>f</sup>	+++ <sup>f</sup>	+++ <sup>f</sup>

<sup>a</sup> The degree of gastric infiltration and/or expression of antigens was scored as mild (+), moderate (++), or severe (+++).

<sup>b</sup> Few scattered cells in mucosa.

<sup>c</sup> Weakly stained cells in follicle coronas.

<sup>d</sup> Scattered cells in mucosa, lamina propria, and epithelium.

<sup>e</sup> Densely labeled cells in lymphoid follicles.

<sup>f</sup> Positive reaction in epithelium and infiltrating leukocytes.

## DISCUSSION

In the present study, we have shown that mice orally immunized with *H. pylori*-derived rUrease were protected from infection upon challenge with living *H. felis* organisms. Oral immunization with rUrease resulted in the generation of secretory IgA antibody, and high levels of intestinal IgA antibody against urease were associated with protection of mice against infection when challenged with living *H. felis*. The high degree of conservation at the amino acid level between *H. pylori* and *H. felis* urease (11) may account for the immunological basis of cross-protection with UreB subunit containing protective epitopes (11a, 26). The ability of rUrease antigen to confer protection in immunized mice against infection with *H. felis* was similar to that afforded by *H. felis* sonicate antigens, as shown in this study and others (2, 6, 22). However, while the protective effect of oral immunization with *H. felis* sonicate antigens has been observed in mice challenged with *H. felis* 3 to 14 days after the last immunization (2, 22), we have found the protective response to last up to 6 weeks postimmunization. The findings reported herein are consistent with previous studies showing induction of mucosal IgA and absence of infection in mice immunized with *H. felis* sonicate (6). Furthermore, the present observations indicate that intestinal anti-urease IgA antibody activated by immunization with rUrease may be required to interfere with the establishment of *H. felis* infection and suggest that low levels of urease-specific IgA, as assayed in *H. felis* sonicate-immunized mice, may be protective as well. However, it is not clear whether IgA antibody responses directed against *H. felis* surface structures other than urease may also interfere with colonization. Detailed analyses of antibody responses to *H. felis* surface antigens in protected mice and studies with urease-negative *Helicobacter* mutants (8, 36) may help answer this question. Because IgA may function in the mucosal environment by inhibition of microbial adherence to epithelial cells (39), the mucosal anti-urease IgA antibody response generated in animals immunized with rUrease or *H.*

*felis* sonicate would have the potential to interfere with the *H. felis*-gastric epithelium interactions which result in colonization. The findings that high levels of IgG antibody induced by parenteral immunization did not protect against infection (3, 9) and that an orally administered anti-*H. felis* MAb protected mice against *H. felis* infection (6) support an important role for local IgA antibody in the prevention of infection in target gastric tissue. The ability of oral immunization to prime the mucosal compartment for a greater IgA antibody response upon deliberate challenge with live *H. felis*, presumably via recognition of urease epitopes displayed at the *H. felis* surface or present as luminal antigen, suggests that protective levels of IgA antibody may be generated in uninfected, immunized hosts as a function of reexposure to the organism.

The continued presence of *H. felis* in the gastric mucosa of PBS-treated mice, as shown in this study, and in gastric tissue of chronically infected mice (12) may signal persistent antigenic stimulation by *H. felis* which gives rise to gastric germinal-center reactions, whereas the reduction or absence of gastric lymphoid follicles in immunized animals may reflect clearance of the organisms and down-regulation of B-cell function. The observation of regression of B-cell gastric lymphoma after eradication of *H. pylori* (40) supports the notion that formation of organized lymphoid tissue in gastric mucosa may be antigen driven. Whether the gastric epithelium overlying mucosal follicles harbors M cells specialized for antigen uptake found in intestinal lymphoid tissues (29) or whether the follicular architecture is maintained by stimulation with luminal *H. felis* antigens which gain access to gastric mucosa is not known at present. However, the finding of scattered CD45R<sup>+</sup> B cells and IgM<sup>+</sup> and IgA<sup>+</sup> B cells in gastric tissue of protected animals raises the possibility that oral immunization with rUrease may result in the accumulation and proliferation in gastric tissue of IgA antibody-secreting cells activated in mucosa-associated lymphoid tissue (38).

Recent studies have suggested that T-cell populations from infected hosts are sequestered into gastric mucosa (20) and may regulate local B-cell function and IgA antibody secretion (12). The finding of discrete T-cell populations in murine gastric mucosa after inoculation with live *H. felis* suggests the recruitment and/or local proliferation of T cells dominated by the CD3<sup>+</sup>, CD4<sup>+</sup> CD8<sup>-</sup>, and  $\alpha\beta$ TCR<sup>+</sup> phenotypes. Although the gastric TCR specificities for *H. felis* antigens in infected or protected mice have not been probed, recent findings have shown the antigen-specific activation of gastric T cells (19). That gastric CD4<sup>+</sup> CD8<sup>-</sup> T cells may play a role in local IgA antibody production is also suggested by findings of increased gastric IgA antibody after oral immunization with *H. felis* antigens (6) or with rUrease (28).

Previous studies have found up-regulation of class II major histocompatibility complex expression by gastric epithelial cells in infected patients (10). Whereas I-A antigen expression by gastric epithelial cells and infiltrating mononuclear leukocytes was shown in the present work, these observations suggest that interaction with, but not necessarily colonization by, *H. felis* may be sufficient for induction of I-A, since no differences in the immunohistochemical expression of I-A antigen were seen between infected and protected mice. While the effects of oral immunization on the activation of immunological effector functions by gastric T cells and B cells are not well understood, the current observations indicate that oral immunization with rUrease antigen interferes with the establishment of infection with *H. felis*.

#### ACKNOWLEDGMENTS

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